

# University of Wollongong - Research Online

## Thesis Collection

Title: Evidence for effects of oat [beta]-glucan on satiety and weight control

Author: Eleanor Beck

Year: 2009

Repository DOI:

### Copyright Warning

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site.

You are reminded of the following: This work is copyright. Apart from any use permitted under the Copyright Act 1968, no part of this work may be reproduced by any process, nor may any other exclusive right be exercised, without the permission of the author. Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material.

Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

**Unless otherwise indicated, the views expressed in this thesis are those of the author and do not necessarily represent the views of the University of Wollongong.**

Research Online is the open access repository for the University of Wollongong. For further information contact the UOW Library: [research-pubs@uow.edu.au](mailto:research-pubs@uow.edu.au)



RESEARCH ONLINE

University of Wollongong  
Research Online

---

University of Wollongong Thesis Collection

University of Wollongong Thesis Collections

---

2009

# Evidence for effects of oat [beta]-glucan on satiety and weight control

Eleanor J. Beck

*University of Wollongong*

---

## Recommended Citation

Beck, Eleanor J., Evidence for effects of oat [beta]-glucan on satiety and weight control, Doctor of Philosophy thesis, School of Health Sciences, Faculty of Health and Behavioural Sciences, University of Wollongong, 2009. <http://ro.uow.edu.au/theses/3188>

Research Online is the open access institutional repository for the University of Wollongong. For further information contact Manager Repository Services: [morgan@uow.edu.au](mailto:morgan@uow.edu.au).



RESEARCH ONLINE

## **NOTE**

This online version of the thesis may have different page formatting and pagination from the paper copy held in the University of Wollongong Library.

## **UNIVERSITY OF WOLLONGONG**

### **COPYRIGHT WARNING**

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site. You are reminded of the following:

Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material. Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

# **Evidence for effects of oat $\beta$ -glucan on satiety and weight control**

A thesis submitted in fulfilment of the requirements for the award of the degree

**Doctor of Philosophy**

from

University of Wollongong

by

**Eleanor Jane Beck**

BSc. (University of Queensland)  
Honours I (University of Queensland)  
Grad. Dip. Nutr. & Diet. (Queensland University of Technology)  
Advanced Accredited Practising Dietitian

**School of Health Sciences**

**2009**

© Copyright 2009

by

Eleanor Jane Beck

All Rights Reserved

## **DECLARATION**

I hereby declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Health Sciences, 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.

---

Eleanor J. Beck

Date:

## **DEDICATION**

To

Mum, Dad and Craig

## **ACKNOWLEDGEMENTS**

I would like to express my sincere gratitude to my supervisors who have helped me along this path. Professor Linda Tapsell has at all times encouraged and supported my career both throughout this thesis and throughout my time at the University and I am greatly appreciative of her time, support and friendship. I would also like to thank Professor Xu-Feng Huang who has opened my eyes to a whole new world of research outside dietetics, and provided wonderful academic guidance and support.

I would like to make special thanks to Dr Marijka Batterham who not only helped with my research through learned statistical advice but has also been a true friend as we procrastinated over our research, teaching and children. I would also like to thank Associate Professor Peter Williams for his encouragement and friendship over the past 10 years which has been of untold benefit to me.

I would also like to thank the research teams at the Centre for Translational Neuroscience and the Smart Foods Centre who have created a happy workplace over the last years. In particular, I would like to thank Serina Faraji for assistance in the intervention trial within this thesis and Greg Teuss for his assistance with the many blood samples my studies always seemed to collect and require analysis of.

I would like to acknowledge the financial support of an Australian Research Council Scholarship which has made research and study a viable option. I would also like to thank Kirsten Grinter from Nestle for invaluable guidance on commercial aspects of this thesis and some great conversations in between. I would also like to acknowledge Melissa Toh for her input in the early product development phases of my research and the assistance of John Pitcher in ensuring trial products were always available as required, no matter how huge the deliveries became and no matter how much storage space this meant I required.

Dr Susan Tosh from Agriculture and Agri-Food Canada has not only helped physically with the  $\beta$ -glucan analyses in this thesis, but her expertise in all things  $\beta$ -glucan has been shared at all times and her friendship has been greatly valued. Ideally the AACC will choose tropical locations for most conferences so we can continue our



friendship and work in beautiful surroundings. Similarly, Ruedi Duss has been at all times helpful in advice on  $\beta$ -glucan, oats and regulations and I appreciate his support, friendship and sometimes his Swiss humour.

Most importantly I wish to thank my family for their unwavering support. This starts with my parents who told me at a young age that I should be so excited to have the opportunity to learn new things. I did not appreciate this back then, but my PhD has given me many opportunities to learn and I am fully appreciative of this. My husband Craig told me I could do this when the children were still aged 2, 4 and 6 and I thought it ridiculous. He told me it would be fine and I should do it. His support, encouragement, love and friendship never waivers, and for this I will always appreciate how lucky I am. Kennedy, Lewis and Finlay are 9, 7 and 5 now and their ability to constantly distract me from my work has made me appreciate them even more. This thesis may not be perfect but it has been enjoyed and nurtured along with my three beautiful cherubs as we have all learned and grown in the last three years.

## LIST OF ABBREVIATIONS

AACC	American Association of Cereal Chemists
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
AUC	Area Under the Curve
$\beta$ -glucan	(1 $\rightarrow$ 3)(1 $\rightarrow$ 4) Beta-D-glucan
BMI	Body Mass Index
CCK	Cholecystokinin
DPPIV	Dipeptidyl peptidase IV
EDTA	Ethylenediaminetetraacetic acid
ES1	Excellent Source of fibre under current regulation
ES2	Excellent Source of fibre under proposed regulation
FDA	Food and Drug Administration
FSANZ	Food Standards Australia New Zealand
GI	Glycaemic Index
GIT	Gastrointestinal Tract
GLP-1	Glucagon-like-peptide-1
GS1	Good Source of fibre under current regulation
GS2	Good Source of fibre under proposed regulation
HBG	High $\beta$ -glucan
HBGO	High $\beta$ -glucan oat bran ingredient

HBGX	High $\beta$ -glucan extracted ingredient
HDL	High Density Lipoprotein
LBG	Low $\beta$ -glucan
LDL	Low Density Lipoprotein
MBG	Medium $\beta$ -glucan
MW	Molecular Weight
NPY	Neuropeptide Y
P293	Proposal 293
PASSCLAIM	Process for the Assessment of Scientific Support of Health Claims on Food
PYY	Peptide Y-Y
PYY <sub>3-36</sub>	Peptide Y-Y 3-36
RCT	Randomised Controlled Trial
RMANOVA	Repeated Measures Analysis of Variance
RTE	Ready-To-Eat
S1	Source of fibre under current regulation
S2	Source of fibre under proposed regulation
SD	Standard Deviation
TFEQ	Three Factor Eating Questionnaire
TG	Triglycerides
TIU	Trypsin Inhibitor Units
VAS	Visual Analogue Scales

## **PUBLICATIONS IN SUPPORT OF THIS THESIS**

### **Published Papers**

Beck, E.J, Tosh, S.M., Tapsell, L.C., Batterham, M.J. and Huang, X-F. (2009) Oat  $\beta$ -glucan increases postprandial cholecystokinin levels, decreases insulin response and extends subjective satiety in overweight subjects. *Molecular Nutrition and Food Research*, 53, 1343-1351.

### **Accepted Papers**

Beck, E.J., Tapsell, L.C., Batterham, M.J., Tosh, S.M. & Huang, X. F. (2009) Increase in PYY levels following oat  $\beta$ -glucan ingestion is dose dependant in overweight adults. Accepted to *Nutrition Research* 22 September 2009.

Beck, E.J., Tapsell, L.C., Batterham, M.J., Tosh, S.M. & Huang, X-F. (2009) Oat  $\beta$ -glucan supplementation does not impact the effectiveness of an energy-restricted weight loss diet in overweight women. Accepted to *British Journal of Nutrition* 10 October 2009.

### **Submitted Papers**

Huang, X-F., Yu, Y., Beck, E., South, T., Chen, J. Li, Y., Batterham, M., Tapsell, L. High oat  $\beta$ -glucan diet increases satiety via activation of the PYY3-36 -Y2 -NPY anorexigenic pathway in diet-induced obese mice. Submitted to *Journal of Cellular Biochemistry* 16 June 2009.

### **Published Abstracts from Oral Presentations**

Beck, E.J., Tapsell, L.C., Tosh, S.M., Batterham, M.J. and Huang, X. (2009) Dietary interventions with fibre ingredients – considerations, measurements and manipulations. *Cereal Foods World*, 54(4) Supplement, A16..

Beck, E.J., Tosh, S.M., Tapsell, L.C., Batterham, M.J. and Huang, X-F. (2008) Characterisation of a functional ingredient requires multiple assessments. *Cereal Foods World*, 54(3) Supplement, A21

Beck, E.J. Tapsell, L.C., Huang, X-F. and Batterham, M.J. (2008) Relationships between 24 hour recalls and fasting measures of hunger and satiety - subjective and biochemical indices. *Nutrition and Dietetics*, 65; (S2) A11.

Beck, E.J., Norman, J.A., Batterham, M.J., Huang, X-F. and Tapsell, L.C. (2007) Dietetic Practice in Appetite Research – acute meal test studies. *Nutrition and Dietetics*, 64; Suppl.1, S49-S51.

Beck, E., Tapsell, L., Batterham, M. and Huang, X-F. Developing Foods with Fibre Effects. 2008 *International Congress of Dietetics*, Yokohama, Japan. (OS-8-1, Sept 8-11).

Huang, X.F., South, T., Beck, E., Batterham, M., Grinter, K. and Tapsell, L. (2008) Diets high in oat-derived beta -glucan promote negative energy regulatory pathway of hypothalamic NPY system in chronic diet-induced obesity in mice, *The 2008 Obesity Society Conference*, Phoenix, USA, P193.

#### **Other Presentations from 2006-2009**

Beck, E.J., Hazlewood, T., MacDonald, R. and Crockett, N. (2006) Team teaching in clinical dietetics – student feedback and student outcomes. *Nutrition and Dietetics*, 63; Suppl. 1, A57-A59.

## TABLE OF CONTENTS

CHAPTER 1	INTRODUCTION: Fibre, health claims & $\beta$ -glucan .....	1
1.1	Introduction.....	2
1.2	Dietary fibre and related definitions .....	3
1.3	Health claims and regulation .....	6
1.4	Levels of Evidence for Health Claims .....	9
1.5	Regulation of satiety and weight control claims.....	11
1.6	Why fibre for satiety and weight management? .....	11
1.7	Fibre and Glycaemic Response.....	14
1.8	Oats and Beta-Glucan – health benefits.....	15
1.8.1	Cholesterol lowering effects .....	16
1.8.2	Glycaemic Response.....	18
1.8.3	Beta-Glucan, Satiety and Weight.....	19
1.9	Viscosity of beta-glucan – factors affecting physiological function .....	26
1.10	Summary .....	27
CHAPTER 2	METHODOLOGY .....	28
2.1	Methodological Framework.....	29
2.2	Measurements of Satiety .....	30
2.2.1	Hormonal Controls.....	30
2.2.2	Subjective measures of satiety .....	37
2.2.3	Dietary Intake.....	39

2.3	Food variables affecting satiety and satiation .....	41
2.4	Meal Test Studies.....	42
2.5	Randomised Controlled Trials .....	43
2.6	Hypothesis.....	44
2.7	Study Design.....	45
2.7.1	Development of $\beta$ -glucan enriched ready-to-eat cereal .....	45
2.7.2	Meal-test study to measure acute satiety .....	46
2.7.3	Satiety and weight control – 3 month human clinical trial .....	47
2.8	Outcome Measurements.....	48
2.9	Significance of research.....	49
CHAPTER 3 DEVELOPMENT OF $\beta$ -GLUCAN ENRICHED CEREALS.....		50
3.1	Introduction.....	51
3.2	Methods.....	53
3.3	Results.....	55
3.4	Discussion.....	61
CHAPTER 4 ACUTE MEAL TEST STUDY WITH CEREALS CONTAINING $\beta$ - GLUCAN.....		62
PART 1 – Acute effects of $\beta$ -glucan enriched foods .....		62
4.1	Introduction.....	63
4.2	Methods.....	65
4.2.1	Recruitment.....	65
4.2.2	Timeframes .....	65

4.2.3	Test foods.....	65
4.2.4	Anthropometric and dietary assessments.....	66
4.2.5	Clinical measurements.....	67
4.2.6	Appetite and hunger assessments.....	68
4.2.7	Statistical Analysis.....	68
4.3	Results.....	69
4.3.1	Subjects.....	69
4.3.2	Analyses of test foods.....	69
4.3.3	Baseline dietary intakes.....	71
4.3.4	Clinical Indices.....	71
4.3.5	Subjective Satiety.....	75
4.3.6	Second Meal Intake.....	76
4.4	Discussion.....	78
PART 2 - PYY responses to varying doses of oat $\beta$ -glucan in humans .....		82
4.5	Introduction.....	83
4.6	Methods.....	84
4.7	Results.....	84
4.8	Discussion.....	88
CHAPTER 5 LONG TERM EFFECTS OF OAT $\beta$ -GLUCAN IN AN ENERGY- RESTRICTED DIETARY INTERVENTION .....		90
5.1	Introduction.....	91
5.2	Methods.....	93



5.2.1	Subjects and Recruitment .....	93
5.2.2	Dietary Intervention .....	95
5.2.3	Clinical Indices .....	99
5.2.4	Statistical Analysis .....	100
5.3	Results .....	100
5.3.1	Baseline Data .....	100
5.3.2	Dietary Intervention .....	101
5.3.3	Clinical Indices .....	105
5.3.4	Subjective satiety .....	108
5.3.5	Product evaluation .....	109
5.4	Discussion .....	111
CHAPTER 6 DEFINITIONS OF FIBRE AND PROPOSAL 293 – EFFECTS ON FOOD LABELLING .....		115
6.1	Introduction .....	116
6.2	Regulation of Food Labelling in Australia: Relationships to fibre definitions...	116
6.3	Food Standards regulation: Insights from the current research .....	118
6.4	Labelling complexities .....	119
6.5	Labelling for food professionals .....	121
6.6	Conclusions .....	122
CHAPTER 7 CONCLUSION .....		123
7.1	Hypothesis .....	124
7.2	Research Design and Evidence Requirements .....	124

7.3	Outcomes and Disease Markers.....	127
7.4	β-glucan in satiety and weight management.....	128
7.5	Future Directions .....	130
REFERENCES .....		132
Appendix 3-1 .....		148
Appendix 3-2 .....		153
Appendix 5-1 .....		162
Appendix 5-2 .....		163

## List of Figures

Figure 2-1 Visual analogue scale questions.....	38
Figure 2-2 Factors affecting satiety and satiation .....	42
Figure 3-1 Varied cereal samples dried overnight.....	56
Figure 3-2 The final product deemed acceptable for use in an acute feeding trial.....	56
Figure 4-1 Size exclusion chromatograms of $\beta$ -glucan MW in the in vitro extracts.....	70
Figure 4-2 Postprandial insulin responses for control and high $\beta$ -glucan cereal for subjects without obvious hyperinsulinaemia. ....	72
Figure 4-3 Postprandial glucose responses for control and high $\beta$ -glucan cereal .....	73
Figure 4-4 CCK response to various levels of $\beta$ -glucan.....	73
Figure 4-5 Average energy intake at lunch meal .....	77
Figure 4-6 PYY responses corrected for baseline.....	87
Figure 4-7 PYY netAUC for each test meal. ....	87
Figure 5-1 Flow diagram of participation in the study .....	94
Figure 7-1 Levels of evidence compared with measured food intake patterns.....	125

## List of Tables

Table 1-1 Currently debated definitions of dietary fibre .....	5
Table 1-2 Comparison of health claim criteria between Australia and the European Union.....	10
Table 1-3 Outcome measures for weight control according to PASSCLAIM .....	11
Table 1-4 $\beta$ -Glucan health claims .....	17
Table 1-5 Studies with measurements relating to satiety and weight control with consumption of $\beta$ -glucan .....	22
Table 2-1 Outcome measurements in acute and long term studies.....	49
Table 3-1 Ingredients included in formulations for testing .....	53
Table 3-2 Proximate nutritional composition of cereal formulations.....	54
Table 3-3 Characterisation of $\beta$ -glucan ingredients, cereal mixes prior to extrusion.....	58
Table 3-4 Characterization of $\beta$ -glucan cereals varying temperatures and shear rates...	59
Table 3-5 Characterization of $\beta$ -glucan cereals developed for acute meal test study.....	60
Table 4-1 Composition and analysis of test meals.....	66
Table 4-2 Physico-chemical characteristics of $\beta$ -glucan in test meals .....	70
Table 4-3 Post prandial insulin responses for 2 and 4 hours for all subjects and excluding subjects with obvious hyperinsulinaemia.....	74
Table 4-4 Post-prandial glucose, ghrelin and CCK responses.....	74
Table 4-5 Visual Analogue Scales Score .....	76
Table 4-6 Dietary intake after each of the five test meals .....	76
Table 4-7 Mean PYY values for $\beta$ -glucan doses over time.....	86

Table 5-1 Food serves for typical dietary study participant .....	96
Table 5-2 Nutrient composition of trial products .....	97
Table 5-3 $\beta$ -glucan and total fibre content of study products. ....	98
Table 5-4 Baseline characteristics of study subjects.....	101
Table 5-5 Reported energy and macronutrient intakes at baseline, mid-point and 3 months,.....	103
Table 5-6 Intake of study products calculated from food records. ....	104
Table 5-7 Average of mid and final results for contribution of $\beta$ -glucan and total fibre by study products. ....	105
Table 5-8 Changes in Clinical indices over time .....	107
Table 5-9 Visual analogue scale P values for one way ANOVA .....	110
Table 6-1 Grams of fibre required for front of packet labelling of fibre sources. ....	118
Table 6-2 Comparisons of number of serves of breads and cereals .....	119
Table 7-1 Target functions and outcome measures affected by $\beta$ -glucan .....	128

## ABSTRACT

Making claims on the health effects of foods currently presents major challenges to nutrition science. As a case in point, oat  $\beta$ -glucan has been shown to deliver a number of health benefits, including an ability to lower cholesterol levels as well as reducing glucose and insulin responses to a meal. These physiological functions are related to the viscosity and solubility of the  $\beta$ -glucan, with the viscosity a function of concentration and molecular weight. Further, despite epidemiological evidence that high fibre diets are associated with lower levels of overweight and obesity in populations, and experimental evidence that fibre will “make you feel fuller for longer”, there is little evidence linking specific fibres with weight control. Changes to regulations governing health claims in Australia and New Zealand are currently under review, and while they reflect European and other regional positions in allowing claims for  $\beta$ -glucan and cholesterol, they do not address other health benefits such as weight loss. This thesis provides a novel approach to evidence based research in food by combining studies in food science, acute meal tests and longer term dietary interventions. The hypothesis examined in this thesis is that overweight individuals following a nutritionally-balanced, energy-restricted diet including oat  $\beta$ -glucan will experience increased satiety and lose more weight than if they followed the same diet without the added  $\beta$ -glucan.

Product development studies examined the effects of extrusion on the important physical attributes of  $\beta$ -glucan included in a ready-to-eat cereal product. It did not prove difficult to produce a cereal that maintained  $\beta$ -glucan at high molecular weight ( $>1$  million) and was viscous at high concentration (up to 5g  $\beta$ -glucan/cereal serve). Extrusion improved solubility which means the effects of downstream processing in this manner are likely to improve the physiological effects of  $\beta$ -glucan in cereals.

Results of a meal-test study with fourteen subjects found that increasing doses of  $\beta$ -glucan up to 5.5 g, decreased insulin levels ( $P=0.011$ ) and increased subjective satiety measured by visual analogue scales ( $P=0.039$ ). Increasing doses of  $\beta$ -glucan were correlated with increased plasma concentrations of cholecystokinin (CCK) and peptide Y-Y (PYY) ( $R^2=0.970$  and  $0.994$  respectively). Food intake at a subsequent meal was decreased with inclusion of  $\beta$ -glucan in the earlier test meal, although the differences were not statistically significant.

A 3-month randomised controlled trial of 66 overweight women was then conducted to investigate the effects of two different doses of  $\beta$ -glucan (5-6g or 8-9g) on weight loss within an energy-restricted regimen. Outcome measures included weight loss and markers of appetite regulation (hormones) as well as changes in metabolic variables related to cardiovascular disease. All groups lost weight (approximately 5% of body weight) and showed a reduced waist circumference ( $P < 0.001$  for both). The study sample also showed reductions in total cholesterol, LDL, HDL, leptin, PYY, glucagon-like-peptide-1 (GLP-1) values and an increase in CCK levels. No significant differences were noted between the groups for all outcome values except fasting PYY levels ( $P = 0.018$ ) but levels did not correlate with increasing dose.

Thus, the addition of oat  $\beta$ -glucan did not enhance the effect of energy restriction on weight loss in mildly overweight women, although large standard deviations in observed results, suggested that individual responsiveness makes elucidation of significant changes difficult. Adding these results to the body of evidence, it seems that some evidence exists relating to  $\beta$ -glucan and satiety with the most likely mechanisms relating to changes in absorption of nutrients and resultant release of anorexigenic hormones. There appears to be insufficient evidence to suggest the validity of a claim related to  $\beta$ -glucan and weight control. Further research of this nature would build on the knowledge of the mechanisms of satiety elucidated here, and this would further investigate how  $\beta$ -glucan and other soluble fibres may help weight control over longer time frames.

## **CHAPTER 1      INTRODUCTION: Fibre, health claims & $\beta$ -glucan**



## 1.1 Introduction

Overweight and obesity is a significant health concern for Australians. The summary of results of the 2007-2008 National Health Survey, released by the Australian Bureau of Statistics in 2009, shows 62% of all Australians are overweight or obese. When compared to results from surveys in 1995, 2001 and 2005 the proportion of adults in these categories is steadily increasing <sup>1</sup>. Overweight and obesity is a risk factor for all cardiovascular diseases, diabetes and certain forms of cancer, and as such, a significant cost burden for the Australian healthcare system.

There is no single cause to overweight and obesity. However, the fundamental issue is a balance between energy intake and energy output. Strategies which can help reduce energy intake are paramount in a society of ubiquitous food supply yet variable nutrient quality. Control of appetite, including the key parameters of satiation (the feeling of fullness allowing meal cessation) and satiety (the amount of time until the next eating episode), may help individuals control food intake and hence manage overweight and obesity.

Fibre intake, by increasing satiety, may play a role in regulation of dietary intake and therefore weight control. Soluble fibres, such as  $\beta$ -glucan in oats, can influence appetite by chemical and physical properties (particularly their bulking action), and increase viscosity in the gastrointestinal tract <sup>2</sup>. Similarly, intakes of whole grains are implicated in weight control <sup>3</sup>. Oats are classified as a 'whole grain' and this together with their high fibre content may explain some of the positive associations of oats with health.

The experimental components of this thesis investigate  $\beta$ -glucan sourced from oats, and links between dose and markers or measures of satiety. These results are then used to determine the validity of the major hypothesis, that overweight individuals following a nutritionally-balanced, energy-restricted diet including oat  $\beta$ -glucan will experience increased satiety and lose more weight than if they followed the same diet without the added  $\beta$ -glucan.

The literature reviewed within this thesis gives an overview of the definitional debate around fibre and its measurement. The specific role  $\beta$ -glucan may play in a healthful diet, including cholesterol, glycaemic and weight control is reviewed. Biochemical and

subjective measures of satiety as well as the role of fibre in satiety and weight control are discussed. Current regulation of health claims is included along with a framework for provision of evidence relating  $\beta$ -glucan and satiety and weight control.

This thesis reviews existing evidence, together with that gathered as part of the experimental component, to examine the possibility of a health claim related to  $\beta$ -glucan, satiety and weight control.

## **1.2 Dietary fibre and related definitions**

Dietary fibre was historically defined on the basis of analytical methods used to measure or extract it from foods (methods described by the Association of Official Analytical Chemists – AOAC) and this has been the definition used in the United States by the Food and Drug Administration (FDA)<sup>4</sup>. De Vries<sup>5</sup> reviews the evolution of dietary fibre from “the nondigestible constituents that make up the plant cell wall” to the early linking of fibre to health and disease prevention in the “fibre hypothesis”<sup>6-9</sup>.

Dietary fibre remained defined as plant components resistant to human digestive hormones but the key element of the hypothesis was the relationship between good health and fibre consumption. Diets high in fibre have been shown to be protective against obesity<sup>10</sup>, cardiovascular disease<sup>11</sup>, diabetes<sup>12</sup> and some types of cancer<sup>13, 14</sup>.

The definition of dietary fibre is contentious, in part because although fibre has obvious beneficial effects such as laxation, blood glucose attenuation and blood lipid improvements, the precise links between diet and disease are not always defined by epidemiological evidence or an individual trial monitoring specific outcomes. So although studies of very high fibre diets created the “dietary fibre hypothesis” it is possible that components of those diets would not be measured by recognised analytical techniques or at least not discussed in the initial research related to fibre. The typical example relates to the original Burkitt work<sup>6</sup> which did not discuss that the high fibre African diets studied would have contained significant resistant starch. This resistant starch would only meet some definitions of fibre currently being debated<sup>15</sup>.

The definitions which have most recently been put forward in this debate are listed below (Table 1.1). De Vries, discussing the American Association of Cereal Chemists (AACC) definition, highlights the importance of the congruence of this definition of fibre with that used over the past 30 years. Importantly, the definition describes the

same plant components as always used. This means that analytical techniques and analysis were still valid and even more critically, for the use of this definition in a commercial food environment, previous research linking dietary fibre with health, or lack of fibre with disease does not change <sup>5</sup>.

The contentions with definitions such as the AACC definition are that these would include resistant starch, oligosaccharides and fibre supplements, yet traditional dietary fibre intake recommendations have been based on intakes of fruit, vegetables and whole grains (initially and to some extent to primarily based on epidemiological research). This means to ascribe the same health effects (generally, effects first described in the 1970's fibre hypothesis) may be erroneous. It does not mean that these components do not provide healthful effects, but that the science measuring quantities and provision of the components is different.

Positions against such definitions argue that health claims or recommendations for the “fibre-like substances which are not naturally occurring” should be substantiated and communicated separately to those of the traditional fibre definition. Hence, it has been argued that fibre is only that which consists of the edible components of plant cell walls <sup>15, 16</sup>. The controversies are relevant to any fibres which are collected, modified, extracted and concentrated for use as this means they may not be delivered in their natural environment and as such may not be classed as “intact” fibre. With any functional component, significant testing in both the delivery food, within the context of the whole diet is required. This identifies the functional components in the ingredients but within the context of how the food would be consumed. Altering a component or ingredient may change health outcomes, but changes can be positive or negative, and can only be measured when all dietary parameters are reviewed.

**Table 1-1 Currently debated definitions of dietary fibre**

<i>Group</i>	<i>Definition</i>
AACC 2000 <sup>17</sup>	“Dietary fibre is the edible parts of plants or analogous carbohydrates resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibres promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation.”
FDA 1987 <sup>4</sup>	Dietary fibre is the material isolated using AOAC INTERNATIONAL Enzymatic-Gravimetric Method 985.29. This method includes lignin and nonstarch polysaccharides and some resistant starch, inulin, chitin, chitosan, chondroitin sulfate, and noncarbohydrate material. This method does not include oligosaccharides, polydextrose, or resistant maltodextrins.
Codex 2008 <sup>18</sup>	Dietary fibre means carbohydrate polymers: 1. with ten or more monomeric units, 2. which are not hydrolysed by the endogenous enzymes in the small intestine of humans and belong to the following categories: edible carbohydrate polymers naturally occurring in the food as consumed; carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities; synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities. (Decision on whether to include carbohydrates from 3 to 9 monomeric units should be left to national authorities.)
European definition 2008 <sup>19</sup>	Definition of the material constituting fibre and methods of analysis as referred to in Article 1(4)(j) For the purposes of this Directive “fibre” means carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories: edible carbohydrate polymers naturally occurring in the food as consumed; edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence; edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence.’
Food and Nutrition Board US (2001) <sup>20</sup>	Dietary fibre consists of non-digestible carbohydrates and lignin intrinsic and intact in plants. Functional fibre is isolated, non-digestible carbohydrates which have beneficial physiological effects in humans. Total fibre is the sum of dietary and functional fibre.
FAO/WHO “Experts” (2006) quoted in <sup>15</sup>	Dietary fibre consists of intrinsic plant cell wall polysaccharides.

Some definitions of dietary fibre distinguish between fibre sources as well as possible health benefits. For example, the definition proposed by the US Food and Nutrition Board <sup>20</sup> describes dietary fibre as endogenous fibre, but includes functional fibres as those which have beneficial physiological effects. However it is not possible to distinguish between physiological effects of multiple fibre sources within a single food. It would be problematic to ascribe separate health effects to fibres in the ingredient

listing if the food was tested as a whole. Defining allowable physiological effects of fibre will also be difficult and require significant effort from scientists and regulators <sup>21</sup>.

The AOAC is currently validating a new method of determination of fibre components in food and this method is currently undergoing inter-laboratory evaluation <sup>22</sup>. The method measures fibres traditionally quantified as well as fructo-oligosaccharides, galacto-oligosaccharides, polydextroses and all of the resistant starches. It will then be possible to label foods with the total fibre values without a summation of separate methods. Comments are currently being sort by Codex Alimentarius <sup>23</sup> in relation to methods of determination for their 2008 definition but presumably the described method will be considered in this review.

In Australia, as in the US, specific definitions of dietary fibre are not necessarily adopted by food regulation authorities (Food Standards Australian New Zealand - FSANZ and FDA respectively) but working definitions refer to methods of measurement acceptable to the AOAC. However, debate within FSANZ and subsequent adoption of maltodextrin and inulin as a fibre <sup>24, 25</sup> has involved research into the likely health effects of these ingredients as well as the possible ramifications for consumers. The definition of dietary fibre currently used by FSANZ as Australia's food regulator shows general consistency with the Codex definition but would be debated by its detractors, and is as follows<sup>26</sup>: *Dietary fibre is that fraction of the edible part of plants or their extracts, or analogous carbohydrates, that are resistant to digestion and absorption in the human small intestine, usually with complete or partial fermentation in the large intestine. The term includes polysaccharides, oligosaccharides (DP>2) and lignins. Dietary fibre promotes one or more of these beneficial physiological effects: laxation, reduction in blood cholesterol and/or modulation of blood glucose.*

### **1.3 Health claims and regulation**

It is accepted that pharmaceutical products can be taken to reduce the risk of disease. For example, cholesterol lowering medication decreases serum cholesterol and in doing so, lowers an individual's risk of associated diseases such as heart attack and stroke. With respect to food, there is common acceptance of dietary guidelines in Australia and overseas, which describe a "healthy diet" leading to a general decrease in risk of disease. Similarly, with specific foods, functional ingredients in food may provide

protection against disease or improvement in symptoms, and specific evidence is required to allow health claims related to this function. In addition, societal beliefs ascribe certain benefits to certain foods or components of food. So in the case of fibre, to claim a food is high in fibre or a source of fibre, conveys to the consumer both the proven benefits or those presumed, even without mentioning these benefits on the product.

Generally speaking, many countries follow the broader guidelines of the Codex Alimentarius for health claims on foods first adopted in 1991. In Australia, health claims are regulated by FSANZ which is currently developing a new food standard for nutrition, health and related claims, proposal 293 (P293), for which a final assessment report was released<sup>27</sup> but awaits final approval after further consultation was requested<sup>28</sup>. Documentation relating to this claim discusses a substantiation framework which will be a requirement for manufacturers before making a claim on a food product<sup>29</sup>. Specifically, claims are divided into general level claims and high level claims. General level health claims (GLHC) include content claims which describe the presence of a nutrient, vitamin or other substance, and diet-health claims which describe the nutrient, vitamin, mineral or other substance in relation to a health effect. High level claims describe a nutrient, vitamin, mineral or other substance in relation to disease or a biomarker of a serious disease<sup>26</sup>.

At present, the following food-disease relationships have been substantiated and are awaiting formalisation<sup>27</sup>:

- dietary intake of calcium, vitamin D status, and reduced risk of persons 65 years and over from developing osteoporosis;
- increased dietary intake of calcium and enhanced bone mineral density;
- reduction in dietary intake of sodium and reduction in blood pressure;
- intake of folic acid in the peri-conceptual period and reduced risk of development of neural tube defects in the foetus;
- reduction in dietary intake of saturated fatty acids and reduction in blood cholesterol, total blood cholesterol, blood low-density lipoprotein (LDL)-

cholesterol, serum LDL cholesterol, total serum cholesterol or serum cholesterol levels;

- reduction in dietary intake of saturated and *trans* unsaturated fatty acids and reduction in blood cholesterol, total blood cholesterol, blood LDL- cholesterol, serum LDL-cholesterol, total serum cholesterol or serum cholesterol levels;
- increased intake of vegetables and fruit and reduced risk of coronary heart disease;
- high intake of vegetables and fruit and reduced risk of coronary heart disease.

Importantly, the acceptance of new health claims related to food will require not just a reference to the health effect or advantage claimed, but also the specific property of the food which provides or enhances this effect. Specific components cannot just be added to foods for benefits or claims made on “unhealthy” foods. For example, manufacturers cannot claim a product has a low glycaemic index (GI) if it has other deleterious effects on individuals whom would normally benefit from low GI foods, such as high levels of saturated fats. The food which is labelled with a general or high level health claim must meet nutrient profiling scoring criteria to assess and overall profile of the food <sup>27</sup>. These scoring criteria are defined as food vehicle eligibility criteria.

The most recent consultation paper <sup>28</sup> for P293 requested by the Ministerial Council outlines the requirements for GLHC to be previously defined in Standard 1.2.7 Nutrition and Health Related Claims. This means that a list of claims pre-approved by FSANZ will be defined and manufacturers can use these claims with evidence that their product contains the described ingredient/s.

In the absence of a consensus on regulation in Europe, the International Life Sciences Institute (ILSI) Europe, coordinated a project to enhance the ability of the European Agri-Food Industry to compete globally in the functional foods market and benefit public health through development of innovative foods suitable to modern lifestyles <sup>30</sup>. The project developed PASSCLAIM – Process for the Assessment of Scientific Support of Health Claims on food. PASSCLAIM had the following objectives:

- to evaluate existing schemes which assess scientific substantiation;

- to produce a generic tool for assessing the scientific support for health claims for foods;
- to establish criteria for markers which can be used to explore the links between diet and health.

The various frameworks for health claims regulation internationally describe GLHC and processes such as PASSCLAIM seek to clarify criteria for higher level claims. The processes can then be used to develop a dossier of evidence related to a particular product or component of food.

#### **1.4 Levels of Evidence for Health Claims**

A recent supplement to the Journal of Nutrition <sup>31</sup> describes the process for substantiation of health claims on food between countries and regions. In order to facilitate trade, common approaches between regions are important, although the wide variation in food supply and public attitudes means that differences will exist. Comparisons between the PASSCLAIM framework and FSANZ's P293 are detailed in Table 1.2 below, showing distinct similarities. Frameworks from other countries such as Japan and China also identify similar requirements for substantiation <sup>32, 33</sup>.

Tapsell <sup>34</sup> describes a co-existence between science, marketing and translation of messages to the public where it is the first of these which is fully translatable between cultures. Even then, because foods will be consumed differently or with different background diets, specific claims are likely to always require individual review between regions. Although critics of "functional foods" exist, there is a congruence of methodologies for development of public health messages such as dietary guidelines and nutrient reference values, and that of health claims <sup>34</sup>. Both recognise background diets, safe levels and usual intake and require substantial review of existing scientific literature, occasionally recognising flaws or omissions which then direct further research within an area. The most common requirements for evidence related to food functionality relate to analysis of the product identifying the ingredient and its stability; the overall nutritional content of the food, scientific evidence related to both the ingredient and food in relation to functional outcome and finally safe levels of consumption <sup>31</sup>.



**Table 1-2 Comparison of health claim criteria between Australia and the European Union**

<i>PASSCLAIM criteria for the scientific substantiation of claims</i> <sup>30</sup>	<i>Examples of evidence from P293 substantiating nutrition, health and related claims on foods</i> <sup>35</sup>
1. The food or food component to which the claimed effect is attributed should be characterized.	Studies should clearly identify/characterize dietary pattern, the specific food/component consumed
2. Substantiation of a claim should be based on human data, primarily from intervention studies the design of which should include the following: 2a. Study groups representative of target group 2b. Appropriate controls 2c. An adequate duration of exposure and follow up to demonstrate the intended effect.	The evidence must be of a suitable quality and level and include appropriate human studies.
2d. Characterization of the study groups' background diet and relevant aspects of lifestyle.	Inclusion of appropriate controls Study duration of sufficient time to allow development of health effect being measured ... (including) sustainability of this outcome.
2e. An amount of the food or food component consistent with its intended pattern of consumption.	Dietary recording techniques used should be validated. Lifestyle practices, age, health status, etc should be reported. The required intake of the food/component should be achievable in the context of the total diet of the intended population group/s.
2f. The influence of the food matrix and dietary context on the functional effect of the component.	Studies constructed using a different matrix to the food matrix in which the component will be found, must consider bioavailability.
2g. Monitoring of subjects' compliance re intake of food/component under test.	Consideration should be given to whether the study participants adhered to the intervention.
2h. The statistical power to test the hypothesis.	Studies of sufficient participants, in test and control groups, to reach confident conclusions of outcome.
3. When the true endpoint of a claimed benefit cannot be measured directly, studies should use markers.	Biomarkers may be used, for example, because of a long time period between exposure and clinical manifestations of disease, or for ethical/cost reasons.
4. Markers should be:- biologically valid in that they have a known relationship to the final outcome and their variability within the target population is known; - methodologically valid with respect to their analytical characteristics.	Where markers of intake or exposure are used, they should be specific to the dietary intervention being measured, measure responses across the range of intakes being studied, be measurable with precision and sufficient sensitivity and be applicable to the population group being studied.
5. Within a study the target variable should change in a statistically significant way and the change should be biologically meaningful for the target group consistent with the claim to be supported.	Assessors of health effects should be trained in applying specific diagnostic or assessment criteria and should be unaware of the exposure status of the participant. Finding of statistical significance in a diet-disease relationship does not automatically imply that a health claim based on this relationship.
6. A claim should be scientifically substantiated by taking into account the totality of the available data and be weighing of the evidence.	Substantiation is deciding whether a body of scientific evidence supports a claimed relationship between a food, property of a food (including a nutrient, bioactive substance or defined property of food) and a specific health effect. This decision is made on the basis of assessment of all available scientific evidence, on a claim-by-claim basis.

## 1.5 Regulation of satiety and weight control claims

In 2004, a PASSCLAIM subgroup published specific criteria for substantiation related to body weight regulation, insulin sensitivity and diabetes risk <sup>36</sup>. This document examines both the links between, and characterisation differences of these diseases. It examines target functions of body fat deposition, insulin sensitivity and blood glucose regulation as well as associated functions of energy intake, energy expenditure, fat storage and oxidation, lipotoxicity, body fat composition, inflammation, oxidative stress, vascular function and glucose production and utilization. For health claims related weight control, the PASSCLAIM group listed specific potential claims <sup>36</sup> which can be used as outcome measures to show the effects of a food or food component (Table 1.3).

**Table 1-3 Outcome measures for weight control according to PASSCLAIM <sup>36</sup>**

<i>Claims based on modifications of the target function</i>	<i>Claims based on modifications of markers of other relevant associated functions</i>
reduces the risk of body weight gains	helps to reduce energy/food intake
contributes to body weight reduction	reduces appetite
decreases body fat	increases satiety
reduces abdominal fat	increases metabolic rate/energy expenditure
	increases lipid oxidation?

The criteria <sup>30</sup> are specific and provide a useful framework for researchers wishing to work in the area of health claims on food. More recently, the European Union adopted legislation in relation to nutrition and health claims on foods citing similar criteria <sup>37</sup>.

## 1.6 Why fibre for satiety and weight management?

Despite definitional, mechanistic and analytical debate, the health benefits of foods with high fibre are quite irrefutable. Foods high in fibre are generally ingested more slowly due to a greater requirement for mastication. Once in the stomach they cause greater stomach distension which in turn acts to increase vagal signals of fullness. Delayed gastric emptying occurs with high fibre foods, therefore increasing gastrointestinal transit time <sup>10, 12</sup>. There is decreased contact of the food bolus with digestive enzymes. This disrupts micelle formation and alters diffusion and interaction of nutrients with the gastrointestinal wall <sup>38</sup>. There is a resultant decrease in macronutrient absorption <sup>2</sup>. These nutrients reach further into the gastrointestinal tract (GIT) inhibiting the gastric

hunger hormone ghrelin, and stimulating the duodenal satiety hormone, cholecystokinin (CCK).

Unabsorbed nutrients in the distal end of the GIT produce an ileal break, causing the further release of CCK, glucagon-like-peptide-1 (GLP-1) and peptide-YY<sub>3-36</sub> (PYY<sub>3-36</sub>), all of which decrease appetite. The high levels of these hormones in turn, cause even slower gastric emptying, ensuring the longer lasting effects of satiety hormones in response to intake of dietary fibre.

This action of all forms of fibre is proposed to explain the evidence at population level, for the benefits of fibre and whole grains containing fibre. Epidemiological evidence links fibre intakes to population levels of overweight and obesity<sup>10</sup>. For example, overweight and obesity rates in the United States, with a dietary fibre intake averaging 15 g/day are four times that of African nations where fibre intakes are up to 80 g/day. It has been documented that individuals with higher fibre intake tend to be leaner than those individuals with lower fibre intake<sup>39</sup>. Similarly, studies show that obese individuals typically consume less fibre than their normal weight counterparts<sup>40-42</sup> and that dietary fibre intake is inversely proportional to measures of adiposity such as subscapular skinfold thickness<sup>43</sup>. Smaller crosssectional studies also identify an association of wholegrain intake and body mass intake, where the participants with lowest body mass index (BMI) consumed the most number of serves of wholegrain<sup>44</sup>.

A prospective study of men aged 40-75 years at baseline in 1986<sup>45</sup>, identified that the consumption of whole grains (typically high fibre foods) was inversely related to weight gain over time. Furthermore, as fibre consumption increased (from fibre enriched whole grains) a further reduction in weight gain was seen in the group. Fibre from fruit and vegetables was also inversely related to weight gain. Similar results have been shown in females<sup>46</sup>. Regardless of their weight at baseline, women who consumed more whole grains consistently gained less weight than those consuming less whole grains.

Critically, dietary fibre intake has been linked not only to body weight and adiposity at a baseline level but seems preventative against weight gain and other CVD risk factors<sup>47</sup>. In the 10 year CARDIA (Coronary Artery Risk Development in Young Adults) study, the researchers found greater associations of high fibre intake with lowered blood

pressure, fasting insulin, triglycerides and LDL cholesterol than was found with fat and saturated fat consumption. This means that over a greater time frame, an individual with greater intake of dietary fibre is likely to weigh less and be leaner, and have less risk of lifestyle diseases such as heart disease, stroke and diabetes<sup>47, 48</sup>. Certainly, precise mechanisms are unclear but the evidence is still overwhelming that a general narrative to increase dietary fibre improves cardiovascular health, and this is in addition to other positive associations such as a decreased risk of colorectal cancers<sup>49, 50</sup>.

In the case of fibres sourced from grain foods, groups investigating the “dietary fibre hypothesis” began researching evidence of ‘whole grain’ effects. Importantly, epidemiological evidence which shows benefits of fibres is difficult to separate from evidence related to whole grains as the populations are usually the same group. Consequently, controversy still exists as to whether benefits result from fibre, whole grains, components such as phytochemicals delivered in foods containing the fibre or grain, or whether it is a synergistic relationship between all three which is beneficial<sup>51</sup>. It is therefore important to recognise that assigning benefits to any singular nutrient or other component may under- or over-state efficacy.

Many early reports linking dietary fibre intake with weight loss in individuals resulted from incidental findings. For example, a general practitioner treating elderly patients for constipation noted good tolerance and improved bowel frequency after instructing patients to consume bran biscuits. Unexpectedly, after including high fibre biscuits in their regular diet, a statistically significant reduction in body weight was noted in both male and female participants<sup>52</sup>.

Results showing weight loss with fibre intake have searched for mechanisms for this effect. In particular, the mechanisms related to fibre increasing gastrointestinal viscosity, have been investigated. Studies with ileostomy patients<sup>53</sup> investigate the amounts of macronutrients which would progress into the large bowel and *in vitro* studies which aim to mimic physiological conditions can measure viscosity. Viscous fibres slow transit from the stomach, but overall, fibres result in increased stool frequency and decrease total gut transit time. Regardless of the precise mechanism, the satiety effect of fibre appears to play a role in short term reduction of energy intake. In Holt’s satiety index of common foods, which describes subjective changes in fullness in subjects, high fibre foods such as oats, score very well<sup>54, 55</sup>.

There is limited evidence linking both improved satiety and weight loss to soluble fibres. Krotkiewski<sup>56</sup> published work on four small studies in proceedings from a Dietary Fibre and Obesity conference in 1985. These early studies identified soluble fibres (mostly from oats) as increasing feelings of fullness and decreasing hunger. Subjects consuming diets supplemented with fibre in three of the four studies showed significantly different weight loss compared to the controls. Two of these studies also controlled energy intake in the controls and study patients, while the third allowed *ad libitum* intake. Krotkiewski's fourth study compared soluble and insoluble fibres and could not detect differences in satiety measures from visual analogue scales (VAS), but the soluble fibre group (fibre sourced from guar gum) had significantly greater weight loss.

Howarth and colleagues<sup>10</sup> summarised results of intervention trials reviewing satiety or decreased hunger, weight loss and also decreased energy intake as a result of higher fibre intake compared to placebo or control groups. The vast majority of studies, while not always controlling for food type and palatability, indicated that subjects are less hungry, lose more weight and tend to have a lower energy intake when dietary fibre is increased. It seems that these effects are greater in subjects who historically have lower fibre intake. It is also interesting that in summarising the available information in 2001, this group concluded that positive effects would be found with either dietary fibre from foods, or that in the form of a supplement. Similarly, the American Dietetic Association<sup>57</sup> recognises the potential requirement of some groups for a fibre supplement, even within the framework of a fibre rich diet.

In summary, there is epidemiological and prospective evidence for the role of fibre in weight control. However, specific evidence for individual fibres in individual subjects is more limited.

## **1.7 Fibre and Glycaemic Response**

Generally, foods high in fibre have a low GI and produce a modest glycaemic response. Studies have shown reduced incidence of diabetes in patients with high fibre intakes<sup>58</sup> and also a reduction in development of diabetes for at-risk patients with dietary intervention, including increasing dietary fibre<sup>59,60</sup>. Diets low in fat and high in complex carbohydrate (of low GI) and protein have been shown to mitigate weight gain

in normal subjects and moderate weight loss in overweight subjects <sup>61</sup>. High fibre foods of low GI show increased satiety, delayed return of hunger or decreased *ad libitum* food intake compared with high GI foods <sup>62</sup>.

Recognition that viscous fibres would improve postprandial glucose excursions occurred long before the specific concept of GI <sup>63</sup>. The mechanisms by which fibre controls glycaemic response are related to the viscosity increase in the small intestine (and delayed gastric emptying) <sup>64</sup> and also decreased contact of the gastric contents with amylase enzymes <sup>12</sup>. The reduction in glycaemic response, measured as GI, varies depending on the food viscous soluble fibres are added to. This indicates the importance of processing and product development in fibre-enriched foods. Similarly, it seems the processing of the active components of fibre may explain the inability to develop dose response relationships between fibre and glycaemic response in some studies <sup>65</sup> yet clearly demonstrated in others <sup>66, 67</sup>. Juntunen et al, determined that insulin responses to whole grain foods were more likely related to the form of the food product rather than just the amount of fibre in the food <sup>68</sup>.

A systemic review of the influence of GI on satiety showed general improvements in satiety with lower GI <sup>69</sup>. In longer term studies where a whole of diet approach must be included it seems impossible to separate terms such as GI from fibre and other food constituents (including protein <sup>70</sup>). This is particularly relevant for food manufacturers wishing to make claims related to body weight and GI as once again a specific food effect may be difficult to identify <sup>69</sup>.

## **1.8 Oats and Beta-Glucan – health benefits**

(1→3),(1→4) Beta-D-glucan ( $\beta$ -glucan) is the polysaccharide soluble fibre in oats. Oat bran is separated by milling from whole oats and correspondingly the amount of  $\beta$ -glucan is concentrated in this preparation. In rolled oats the  $\beta$ -glucan is approximately 4%, compared to 5.5-9% in regular oat bran <sup>71</sup>. Increased concentration of  $\beta$ -glucan in oat bran can be achieved by selection of specific oat populations <sup>72</sup> and fine grinding and sieving <sup>73</sup>. More recent purifications of  $\beta$ -glucan have used extraction (Viscofibre™), enzymatic methodologies (Oatvantage™) and specialised milling processes (OatWell™) to create products to specifically deliver  $\beta$ -glucan as a fibre supplement in foods.

The physiological functions of soluble fibre from oats include effects of improving blood lipid profiles,<sup>74-80</sup> and decreasing glycaemic response to a carbohydrate load consumed with the  $\beta$ -glucan<sup>64, 66-68</sup>. Independent effects on appetite or those mediated via glycaemic effects are less well established. However, it is likely that all relate in part to increased gastrointestinal viscosity so it is important to review all these physiological functions.

### 1.8.1 Cholesterol lowering effects

The most publicly well known health effect of  $\beta$ -glucan is the ability of oat bran and oat gum to reduce lipid absorption. The increased viscosity of gut contents causes decreased emulsification by both decreased contractional movements of the gut and also decreased contact with the absorbing surface of the gut<sup>71</sup>. Kerckhoffs et al<sup>79</sup> summarized the mechanisms of action of  $\beta$ -glucan as: effects of decreased surface area of luminal contents; decreased binding of bile acids leading to decreased circulation of bile acids between the gut and the liver and hence increased breakdown of cholesterol to bile acids; and finally lowered glycaemic response decreasing insulin and subsequent hepatic cholesterol production. Similarly, Theuwissen and Jones summarize the likely mechanisms by which all soluble fibres are hypocholesterolaemic<sup>81, 82</sup>. Soluble fibres lower reabsorption of bile acids, hepatic conversion of bile acids increases and ultimately greater quantities of low density lipoprotein (LDL) will be taken up by the liver. Experimentally, these mechanisms have been confirmed in a study on ileostomy patients, showing increased excretion of bile acids on ingestion of oat bran<sup>83</sup>.

Although many studies show lowered serum cholesterol and often specific reduction of low density lipoprotein with ingestion of oats, and sometimes specifically  $\beta$ -glucan, some studies have failed to demonstrate a dose response<sup>65, 76, 78, 84</sup>. Despite some conflicting results, in 1997 the United States FDA approved a health claim that “a diet high in soluble fiber from whole oats (oat bran, oatmeal and oat flour) and low in saturated fat and cholesterol may reduce the risk of heart disease”. The level of 3 g/day consumption of  $\beta$ -glucan is suggested to be of clinical significance. More recently, attention has been paid to the sources (oats, yeast, barley) of  $\beta$ -glucan which may be most likely to be physiologically active and perhaps more importantly, how processing of foods providing  $\beta$ -glucan may alter the viscosity of the  $\beta$ -glucan and therefore reduce or increase the positive effects. The FDA in approving claims on individual foods,

specifies the sourcing and processing of the  $\beta$ -glucan. The parameters of key concern are discussed in the “viscosity” section below.

Examples of the specific health claims related to  $\beta$ -glucan in oats which exist in the United States, United Kingdom and parts of Europe and are summarised in Table 1.4.

**Table 1-4  $\beta$ -Glucan health claims**

<b>Country</b>	<b>Health Claim</b>
United States	Diets low in saturated fat and cholesterol that include 3 g of soluble fiber from whole oats per day may reduce the risk of heart disease.
United Kingdom	The inclusion of oats as part of a diet low in saturated fat and a healthy lifestyle can help reduce blood cholesterol.
Sweden	Soluble fibre from oat bran may help reduce cholesterol.
France	Oat bran $\beta$ -glucan reduces blood glucose and insulin response.
	Consumption of oat bran containing foods and other eligible oat sources containing oat soluble fibers(oat $\beta$ -glucan), as part of a balanced diet, without excess of particularly saturated fats, and physical exercise together help to reduce your cholesterol.
Netherlands	Daily consumption of 1-4 servings of OatWell™ cereal products (providing at least 3g oat $\beta$ -glucan in all) – as part of a diet reduced in cholesterol and saturated fat – can be expected to lower the serum concentration of total and LDL cholesterol in persons with moderately elevated cholesterol levels by on average 4 and 6% respectively, after 5 weeks.

As part of the most recent consultation on P293 in Australia, FSANZ has considered existing evidence in relation to GLHC’s, and the following in relation to  $\beta$ -glucan is included in the draft Schedule 2 of the Standard <sup>28</sup>:

#### *Beta glucan*

*FSANZ previously reviewed the evidence for a high level health claim relationship for wholegrains and heart disease. The relationship was not approved as a high level healthclaim because much of the data in the review related to soluble fibres from specific grains rather than from all wholegrains. Because of previous work, FSANZ gave consideration to the possibility of a GLHC relationship also noting that claims for beta glucan are in the marketplace. FSANZ considers that the evidence between dietary and biliary cholesterol absorption and beta glucan from oats and barley is appropriate to approve a GLHC. A similar claim for soluble fibre (principally beta glucan) and heart disease is recognised in the US health claims regulations and this was used to adapt the conditions for use of the claim.*



### 1.8.2 Glycaemic Response

As previously discussed, foods high in fibre tend to have low GI and reduce blood glucose response and insulin release, in both whole of diet and individual meal test studies. In acute studies, results show decreased rise in blood glucose when meals containing  $\beta$ -glucan are compared to those without<sup>65, 66, 85</sup>. Other studies identified only decreases in insulin<sup>64, 68</sup>. Reviewing various doses of  $\beta$ -glucan given with or without doses of resistant starch, Behall and colleagues showed the greatest decrease in glucose and insulin response with doses of 2.3g  $\beta$ -glucan and 5.06g of resistant starch combined<sup>86</sup> (the highest doses in the study.) The study showed greater decreases in insulin responses with the  $\beta$ -glucan and greater glucose reductions with the resistant starch.

Studies over greater time frames (eight to twenty-three weeks) have shown decreases in fasting glucose and insulin<sup>65, 87</sup>. As described for the effects of soluble fibre in general, postulated mechanisms for glycaemic control by  $\beta$ -glucan include those mediated by slower gastric emptying as well as the presence of nutrients in the distal GIT providing negative feedback for hormonal responses to food ingestion. Battilana et al<sup>64</sup> designed a study to detect mechanisms of action of  $\beta$ -glucan in glucose metabolism. This involved administration of small frequent meals (hourly over 9 hours) to ensure that intestinal absorption was independent of a delayed gastric emptying due solely to viscosity. The results showed delayed carbohydrate metabolism causes decreased glucose concentrations rather than effects of fermentation products in the colon causing *de novo* lipogenesis.

Studies to identify the functional component in glycaemic control by  $\beta$ -glucan sources have shown that the native plant cell wall as found in oat bran or isolates in oat gum have similar effects in healthy subjects or those with Type 2 diabetes<sup>85</sup>. In addition, cereals and cereal bars enriched with  $\beta$ -glucan still show significant decreases in the glycaemic index compared to controls or lower dose oat bran cereals<sup>88</sup> and use of oat bran extracts has been shown to decrease glucagon secretion in response to a meal, adding to the minimisation of glucose excursions<sup>89</sup>. It is likely therefore, that some level of functionality is likely to exist in varied sources of oat  $\beta$ -glucan incorporated into a variety of foods. This is despite evidence that actions are affected by the food in which the fibre is incorporated into<sup>68, 90</sup>.

Specifically,  $\beta$ -glucan lowers glycaemic response by up to 50% when a product of 8-10%  $\beta$ -glucan is consumed and indicates the fibre in naturally occurring forms should be adequate <sup>91</sup>. The later study of Jenkins showed that for each gram of extracted  $\beta$ -glucan tested with a 50g carbohydrate portion, a decrease in GI of 4 units could be anticipated. Responses of insulin and glucose seem to vary based on concentration, preparation and storage of the  $\beta$ -glucan delivery foods <sup>92</sup> although achievement of GI reduction similar to that found by the earlier researchers is possible. Generally, the GI and insulin index measured within a single meal test, do not alter in a parallel manner <sup>92</sup>, <sup>93</sup> but both may be important in the aetiology of lifestyle diseases such as type 2 diabetes and obesity or in its treatment.

Use of  $\beta$ -glucan enriched cereals as part of a low GI breakfast has been shown to allow good glycaemic control, with alterations in lipid metabolism resulting in decreased total cholesterol <sup>94</sup>. It is therefore possible that therapeutic advantages of incorporating  $\beta$ -glucan into the diet for the prevention and treatment of diseases in the “metabolic syndrome” cluster relate directly and indirectly to glycaemic or insulin indices.

### 1.8.3 Beta-Glucan, Satiety and Weight

Studies with the specific outcome of weight loss with inclusion of dietary  $\beta$ -glucan are limited, although the satiety value is relatively well accepted.

In a study comparing supplementation of fermentable ( $\beta$ -glucan and pectin) with non-fermentable (methylcellulose) fibres in conjunction with an ad libitum diet, no weight loss was seen <sup>95</sup>. This study showed greater satiety with the methylcellulose than the  $\beta$ -glucan although neither was linked to a change in energy intake. Similarly inclusion of only 2g of  $\beta$ -glucan in a test meal decreased glucose response but did not alter satiety <sup>96</sup> and so it would seem low doses at a single meal may have limited effects. Other viscous fibres included in cereal bars have been linked to increased feelings of fullness in patients with type 2 diabetes in acute studies <sup>97</sup> but it is critical to recognise that fullness at a particular meal does not necessarily translate to greater satiety throughout the day and neither of these necessarily link to dietary intake.

Animal studies monitoring body temperature show a greater increase in core temperature while rats consume viscous products compared to low viscosity equicaloric foods/drinks <sup>98</sup> and so the pursuit of a palatable viscous dietary supplement has

persisted. Keogh and colleagues recently demonstrated that in humans despite decreased glucose and insulin responses to a high  $\beta$ -glucan diet, the thermic responses between this diet and lower fibre diet could not be discriminated over a 6-hour period, and there were no significant differences in subsequent energy intake<sup>99</sup>.

Rytter<sup>87</sup> showed significant weight loss including an oat-based liquid food in a energy-restricted dietary regimen. The drink contained 4g of dietary fibre although the exact amount of  $\beta$ -glucan was not specified. Subjects lost approximately 6 kg over 23 weeks, although most of this (5 kg) was in the first six weeks. In addition, there was no control group of energy-restricted patients not including the oat product so the results do not infer improved weight loss due to  $\beta$ -glucan. However, the food was well tolerated and the majority of subjects attributed the weight loss to increased feelings of satiety.

Saltzman et al<sup>100</sup> showed no real benefits of including relatively large amounts of oats in a hypocaloric diet, including no significant weight loss compared to control or improved satiety. However, the subjects were only supplied with rolled oats (approximately 90 g/day – equivalent to 3-4g  $\beta$ -glucan spread over the day) equating to only moderate doses at any one time. The amount, molecular weight and therefore final viscosity of  $\beta$ -glucan are not specified but are presumably high in the unprocessed oats, however, the solubility is most likely low.

Recently, a pilot study using oat and barley sourced  $\beta$ -glucan as a dietary supplement in a weight reducing diet, showed weight loss, increased fasting PYY and GLP-1 as well as increased satiety in a meal test after 14 weeks of supplementation<sup>101</sup>. Unfortunately, the 7 subjects were not matched with control subjects also on a weight control diet, so once again the effects cannot be definitively attributed to the  $\beta$ -glucan but may be a more generalised effect of weight loss.

Finally, a small study utilising  $\beta$ -glucan as a fat replacer showed that after 4 weeks, subjects on a “diabetic” diet, lost more weight with improved glycosylated haemoglobin and HDL, than subjects who were only following the standard diet without the supplementary foods<sup>102</sup>. The only other studies specifically targeting weight loss are the 1985 studies by Krotkiewski discussed previously, which showed significant weight loss and in some studies, increased satiety measured by VAS<sup>56</sup>. Table 1.5 summarises the existing studies with outcomes measured relating to satiety and weight control.

Specifically in relation to  $\beta$ -glucan, satiety and weight control, the European Foods Safety Authority, recently sought application on health claims which would be pre-approved under Article 13 of the regulation of Nutrition and Health claims. Those related to  $\beta$ -glucan and satiety and weight control are listed below:

- Consumption of oats causes satiety or consumption of oats can help in weight control due to the satiating effect
- Oat  $\beta$ -glucan - increases satiety/prolongs satiety.

These claims are not yet approved, but notice on these applications should be given by 2010.

In designing research to identify how  $\beta$ -glucan may affect both target functions and markers related to weight control, the PASSCLAIM outcome measures described in Table 1.3 previously, provide a useful framework for research and were used in the original development of research projects related to  $\beta$ -glucan in this thesis. It is unlikely a single clinical trial or even a small group of projects related to a topic can provide substantiation of a health claim. However, provision of a framework gives direction for literature review, research management and analysis of results ensuring a wide variety of outcome measures from a myriad of sources.

**Table 1-5 Studies with measurements relating to satiety and weight control with consumption of  $\beta$ -glucan**

The study outcomes are summarised using the following outcome measures and markers of appetite and weight control <sup>36</sup> described in the PASSCLAIM framework for substantiation of health claims – 1 = reduces the risk of body weight gain, 2 = contributes to body weight reduction, 3 = decreases body fat, 4 = reduces abdominal fat, 5 = helps to reduce energy/food intake, 6 = reduces appetite, 7 = increases satiety, 8 = increases metabolic rate/energy expenditure, 9 = increases lipid oxidation.

Study	Study Design	Study subjects	Time frame	Amount of $\beta$ -glucan	Defined MW, viscosity, solubility	Outcome measures*	Outcomes related to weight/satiety*	Other outcomes
Reyna et al, 2003 <sup>102</sup>	Randomised control trial, $\beta$ -glucan as a fat replacer with artificial sweeteners also, control of ADA diet	16 male, type 2 DM (8/group)	4 weeks	Not defined	no	2 – weight HbA1c, fasting glucose, lipids.	2- yes	Dec HbA1c, improved lipid profile
Howarth et al, 2003 <sup>95</sup>	Crossover pilot intervention, fermentable ( $\beta$ -glucan, pectin) vs non-fermentable fibre, no energy restriction	11 healthy normal or overweight (7 female)	3 wks each diet with 4 wk washout,	9g oat BG+ 18g pectin vs 27g methylcellulose	no	1,2,3 5- 3X24 hr recalls each diet 6,7- VAS each evening + after meal test	1,2,3-no 5-no 6-no 7-methylcellulose better than BG/pectin	Trends with methylcellulose for 5.
Saltzman et al, 2001 <sup>100</sup>	Randomised control trial, oats vs low soluble fibre	41 healthy, normal/overwt (21 oat group approx. equal male/female)	2 wk introduction, 6 wk energy deficit, f-up over 6 mths	45g oats/1000 kcal (80-130g) vs wheat	no	2,3 – underwater weighing, 6,7-frequency of hunger, satiety Dietary compliance	2,3-no 6,7- no but trend to improvement with oats	Trend to better compliance with oats
Pick et al, 1996 <sup>103</sup>	Crossover design	8 men, type 2 DM	24 weeks (12 wks/diet)	9g	no	5 – 48 hour recalls used, glucose, insulin 8 hr profiles at 0,12,24 weeks + lipids	5-no	Improved lipidemic, insulinemic and glycemic responses

\*1. reduces the risk of body weight gain, 2. contributes to body weight reduction, 3. decreases body fat, 4. reduces abdominal fat, 5. helps to reduce energy/food intake, 6. reduces appetite, 7. increases satiety, 8. increases metabolic rate/energy expenditure, 9. increases lipid oxidation.

Study	Study Design	Study subjects	Time frame	Amount of $\beta$ -glucan	Defined MW, viscosity, solubility	Outcome measures*	Outcomes related to weight/satiety*	Other outcomes
Krotkiewski, 1985 <sup>56</sup>	Crossover design, oat bran vs Metamucil, no caloric restriction	32 subjects	4 weeks each diet	5g oat bran/day	no	Constipation symptoms, iron absorption	2-yes although not designed for this outcome	No differences in other outcome measures
	Control trial, oat bran biscuits with caloric restriction vs restriction alone	14 obese women	50 weeks	8g added fibre/day	no	2,3-weight body fat not described 6,7-described as hunger scores	2-yes, 3 no 6,7-yes	
	Intervention with bran biscuits with caloric restriction vs restriction alone	122 obese women	56 weeks	8g added fibre from oat bran	no	2,3,4,6,7 as above, constipation	2-yes, 4-no, 6,7-yes 3 not reported	Decreased constipation with oat bran biscuits; Better compliance in oat bran group
Valle-Jones, 1985 <sup>52</sup>	Intervention, no control, use of oat bran biscuits b.d.	50 elderly subjects (32 female)	12 weeks	Not specified	no	Symptoms of constipation, 2-weight recorded	2-yes	Improvement in symptoms of constipation
Peters et al, 2008 <sup>104</sup>	Randomised crossover, barley $\beta$ -glucan, FOS	21 healthy, BMI avge 25.9	Meal test studies over 2 days	0.9 g $\beta$ -glucan alone or with FOS	no, but measured gastric viscosity increased by $\beta$ -glucan	5- food intake over 2 days 6,7-subjective appetite ratings	5,6,7-no	Increased GIT viscosity with $\beta$ -glucan
Juononen et al, 2009 <sup>105</sup>	Randomised crossover meal test study	20 healthy weight (16 female)	Meal test	10g high viscosity (HV) cf 10g low viscosity (LV)	yes		5,7 HV- yes 6-no	HV improved glycemic and insulinemic responses, LV increased CCK, PYY, GLP-1 responses to meal

\*1. reduces the risk of body weight gain, 2.contributes to body weight reduction, 3. decreases body fat, 4. reduces abdominal fat, 5. helps to reduce energy/food intake, 6. reduces appetite, 7. increases satiety, 8. increases metabolic rate/energy expenditure, 9. increases lipid oxidation.

Study	Study Design	Study subjects	Time frame	Amount of $\beta$ -glucan	Defined MW, viscosity, solubility	Outcome measures*	Outcomes related to weight/satiety*	Other outcomes
Greenway et al 2007 <sup>101</sup>	Intervention pilot, no control	7 with BMI 25-35, 6 female	16 weeks	4 g	Stated high viscosity, high MW, values not reported	2-weight loss 6,7- VAS at 1hr in meal test 0/14 wks, PYY, GLP-1	2,7=yes; 6=no	Inc. fasting PYY and GLP-1
Keogh et al, 2007 <sup>99</sup>	Randomised crossover barley $\beta$ -glucan	14 female, healthy BMI 21-28	Meal test study	10.8g over 2 meals	no		5,6,7,8=no	Improved glycemic and insulinemic responses
Hlebowicz et al, 2009 <sup>106</sup>	Randomised crossover meal test study	12 healthy weight (4 women)	Meal test, yoghurt and muesli with BG or no BG	4g	no	7-satiety scores at 15,90 mins Glycemic changes, gastric emptying rate	7=no	Dec. glycemia with BG
Kim et al, 2006 <sup>96</sup>	Randomised crossover meal test study	19 overweight subjects (10 female)	Meal test glucose vs wheat vs wheat/barley vs barley	0, 1 or 2 g	no	5- subsequent meal intake, 6,7- VAS measures of fullness over 2 hours, hunger, glycaemic response	5=no 6,7=no	Decreased glycaemic response with 2g $\beta$ -glucan in women not men
Berti et al, 2005 <sup>55</sup>	Randomised crossover meal test studies, pre-load of test food, ad libitum meal	15 bread study, 14 pasta study healthy males	Meal test with oat bread vs white bread; oat vs buckwheat vs wheat pasta	Not specified	no	5,6,7-measures of pre-load quantity to satiation + ad libitum intake	5,6,7=yes for oat bread 6,7=yes for oat pasta	Overall pasta and bread are not satiating products.

\*1. reduces the risk of body weight gain, 2.contributes to body weight reduction, 3. decreases body fat, 4. reduces abdominal fat, 5. helps to reduce energy/food intake, 6. reduces appetite, 7. increases satiety, 8. increases metabolic rate/energy expenditure, 9. increases lipid oxidation.

Study	Study Design	Study subjects	Time frame	Amount of $\beta$ -glucan	Defined MW, viscosity, solubility	Outcome measures*	Outcomes related to weight/satiety*	Other outcomes
Rytter et al, 1996 <sup>87</sup>	Weight reduction intervention, no control	31 subjects	Weight reduction 23weeks with no control	Not specified, 4 g total fibre	Viscosity only	2-weight loss 7-survey related to oat product Insulin, enterostatin, lipids	2-yes but no control 7-subjects reported fullness aiding dietary compliance	Decreased chol,LDL, trigs, increased HDL, decreased insulin
Juntunen et al, 2002 <sup>68</sup>	Randomised crossover meal test study	20 healthy weight (10 female)	Meal test with rye bread, rye bread + $\beta$ -glucan, wheat pasta, white bread	5.4g (1.4g in rye bread also)	yes, MW dec'd to 250 000 in bread.	Glycaemic responses over 3 hours, insulin, GIP, GLP-1	Measured hormones only	Increased GLP-1 with $\beta$ -glucan
Bae et al, 2009	Randomised control animal study with $\beta$ -glucan of varied MW, high fat diet control	Animal study with mice	6 weeks	Not defined	yes		1,5,6-yes	

\*1. reduces the risk of body weight gain, 2.contributes to body weight reduction, 3. decreases body fat, 4. reduces abdominal fat, 5. helps to reduce energy/food intake, 6. reduces appetite, 7. increases satiety, 8. increases metabolic rate/energy expenditure, 9. increases lipid oxidation.



### 1.9 Viscosity of beta-glucan – factors affecting physiological function

Some results investigating physiological actions of  $\beta$ -glucan to decrease glycaemic response or serum cholesterol have shown negative results or failed to demonstrate dose responsiveness. It seems the primary actions of  $\beta$ -glucan to improve these parameters are reliant on the viscosity of the  $\beta$ -glucan in the final food product. Viscosity is related to the molecular weight (MW) of the  $\beta$ -glucan, its concentration in solution and in some instances the solubility of the product in the food and GIT environment (that is, water solubility at 37 degrees Celsius). In particular, a high MW  $\beta$ -glucan molecule can entangle to form viscous and pseudoplastic solutions<sup>107</sup>. A decreasing MW and viscosity showed a decreased capability of  $\beta$ -glucan to reduce post-prandial glucose responses<sup>108</sup>.

Although studies listed characteristics of MW, concentration, solubility and viscosity as important as early as the 1990's, even recent studies<sup>109</sup> reporting poor results in cholesterol lowering trials do not report the MW or viscosity of their  $\beta$ -glucan source. Other studies do discuss these characteristics, including the varied solubility of products used as a  $\beta$ -glucan source as a reason a dose response may not be seen<sup>65</sup>. Processes which may decrease MW, solubility and viscosity include temperature, extrusion, storage conditions (such as  $\beta$ -glucanases in flour sources used in food production)<sup>107, 110, 111</sup> and other enzymatic or chemical extraction processes used in modern food technology (summarised in<sup>107</sup>). In addition, while high solubility will not ensure high viscosity, it must be maintained to allow the formation of viscous solutions in the GIT.

Fibres such as  $\beta$ -glucan exhibit a shear thinning behaviour, necessitating that viscosity measures must be quoted for a specific shear rate<sup>112</sup>. Therefore, identifying that the logarithmic value of concentration and MW relate to physiological behaviours of the fibre, gives a framework for development of functional  $\beta$ -glucan foods. However, it should not be forgotten that food form and even storage conditions will be critical in assigning any true physiological effects and therefore potential health claims<sup>90, 113</sup>.

Studies to identify the ideal concentrations, MW and solubility have shown that greater MW produces greater reductions in peak plasma glucose when the  $\beta$ -glucan is incorporated into muffins<sup>114</sup>. A higher dose also had this effect. Solubility was slightly

greater at lower MW (2 200 000 down to 400 000) but at lower weights (120 000) the solubility was decreased. A similar study showed that a freeze-thaw processing of muffins attenuates hypoglycemic effects through decreased solubility <sup>111</sup>.

### **1.10 Summary**

Definitions of dietary fibre are contentious, but in the case of  $\beta$ -glucan significant evidence exists as to its health benefits related to control of glycaemia and hyperlipidaemias and these health promoting effects add weight to its use as a functional ingredient. There is some evidence related to satiety and indications rather than consistent evidence that  $\beta$ -glucan may be useful in weight-reducing diets. In reviewing health effects using high  $\beta$ -glucan ingredients, the active component must always be carefully defined together with its impact on existing dietary patterns, as is the case in all regulatory frameworks. Research relating to overweight and obesity must be carried out in these populations and doses must be realistic and sustainable. A useful framework for gathering significant evidence related to  $\beta$ -glucan is the use of the PASSCLAIM criteria, which outlines outcome measures of weight control together with markers related to appetite.

Given the emerging mechanistic evidence supporting the link between viscosity and its general physiological actions (including glycaemic control and lipid lowering), the role of  $\beta$ -glucan (in its active, high MW and viscous form) in satiety and weight management requires further investigation. Specifically, this research needs to review the role of  $\beta$ -glucan in a whole of diet context, review mechanisms through investigation of biochemical and subjective measures of satiety and measure a final outcome of decreasing body weight or preventing weight gain.

## **CHAPTER 2      METHODOLOGY**

## 2.1 Methodological Framework

The general framework for this thesis rests with the science of proving the specific health effects of food. This framework revolved initially around the discussed outcome measures and markers for weight control discussed in the PASSCLAIM criteria<sup>36</sup>.

These (or similar) are the claims related satiety and weight control which are most likely to be allowed under all regulatory frameworks, given the general consistency in health claim methodologies across Europe and Australia. However, specific to  $\beta$ -glucan there are a number of steps required before reaching a goal of health claims related to weight management. Most importantly, it is necessary to

- clarify that the product under investigation contains functional components;
- to investigate proposed mechanisms such as acute satiety and related hormonal responses;
- to review the functional components within a controlled dietary regimen in humans.

The mechanisms of action of  $\beta$ -glucan are related to an ability to form a viscous bolus within the GIT<sup>90</sup>. This means that the first goal of any research related to  $\beta$ -glucan must be to use a  $\beta$ -glucan source incorporated into a product which will be of sufficient concentration, MW and solubility to allow high viscosity in the GIT under physiological conditions. The first investigation in this thesis relates to development of ready-to-eat (RTE) cereals using extrusion as a processing technology. Ultimately research related to food should have commercial application and so product development is important for both mechanistic and organoleptic parameters.

The second group of investigations in this thesis relate to satiety and investigation of mechanisms including relationships between glycaemic and insulinaemic responses. To demonstrate satiety it may be important to define the specific mechanisms such as altered intestinal transit which alters hormonal responses. Finding a specific pathway may help define the physico-chemical parameters necessary for useful product development but may also link fibres such as  $\beta$ -glucan to other components or ingredients likely to improve satiety alone or in synergy with  $\beta$ -glucan. Measurement

of subjective satiety together with food intake data is of course critical to link any satiety measures with positive outcomes in longer term weight loss interventions. The relative usefulness of hormone markers of weight and satiety are therefore reviewed here, together with the benefits or shortcomings of subjective measures of satiety and food intake measurement methodologies. Putting this together in an appropriate experimental context is the final methodological consideration. The last part of this chapter discusses meal test and clinical trial methodologies for this purpose.

## **2.2 Measurements of Satiety**

Satiety is most commonly defined as the length of time between “meals” or episodes of eating. Satiety tries to define something that makes an individual feel full and potentially decrease intake over a longer time period. Satiation is defined as the feeling to terminate a single episode of eating. For appetite and obesity researchers, investigating foods which improve both satiety and satiation are important, and it may be true that a food which improves either of these also improves the other. The key measurements of satiety are biochemical measures of hormones, subjective measurements and food intake data.

### **2.2.1 Hormonal Controls**

Hormonal controls of satiety are of particular interest to food manufacturers who may be interested in developing and marketing products related to weight management. Ghrelin, CCK, GLP-1 and PYY and its fraction PYY<sub>3-36</sub> are considered the key hormones in the acute regulation of dietary intake. Insulin and leptin are related to homeostasis of energy storage in the longer term, and evidence exists that ghrelin also meets these criteria. Importantly, although changes in these hormones do not directly show altered satiety, measurement helps to elucidate mechanisms which may be targeted to increase satiety, decrease food intake and ultimately decrease the measurements of target functions of weight control.

#### **2.2.1.1 Cholecystokinin**

CCK is a hormone released from the small intestine in response to meals which contain fat. CCK is produced in the duodenum, jejunum and proximal ileum as well as the

myenteric plexus and some areas of the brain <sup>115</sup>. CCK acts in the vagal complex in the brain stem and plays an important role in satiation, that is, the termination of a meal. CCK receptors in vagal nerves which innervate the duodenum are stimulated by CCK release on food entry into the duodenum as well as CCK receptors in the stomach acting to decrease gastric emptying. Gastric filling/distension is required for the satiating effect of CCK <sup>116</sup> so as an inhibitor of gastric emptying some CCK effects may be indirectly mediated by this effect on stomach distention, as well as delayed nutrient progression to the small intestine <sup>117</sup>.

Burton-Freeman <sup>118</sup> investigated the role of various fatty-acid subtypes in influencing CCK release. The link between dietary fat content of a meal and level of CCK was confirmed. Levels of CCK were increased with meals with high levels of polyunsaturated fat from nuts, but this seems to be related to the fibre content of these meals. Burton Freeman <sup>119</sup>, showed that in women at least, increasing the fat or fibre content of a low-fat, low-fibre meal increased the feeling of satiety associated with increased CCK release. The mechanism relates to a slower decrease in lipid from the small intestine, most likely due to increasing viscosity of the gastrointestinal contents due to the fibre. Elevated CCK after higher fibre meals has also been shown in men <sup>120</sup>. In particular, this difference existed in a test meal situation, even when high fibre meals were relatively low in fat.

Of interest to researchers of obesity and related disorders such as diabetes, is the effect of CCK to mediate glucose and insulin responses to meals <sup>115, 121</sup>. It is possible that satiating effects of CCK are in part due to its effect on glucose homeostasis. These links between fibre and CCK and the already documented links between  $\beta$ -glucan and glucose homeostasis mean CCK is a logical hormone to measure in review of mechanisms of  $\beta$ -glucan in relation to satiety.

#### 2.2.1.2 Peptide YY and YY<sub>3-36</sub>

PYY belongs to the pancreatic polypeptide family which includes pancreatic polypeptide and neuropeptide-Y (NPY). All three peptides share a common tertiary structure. PYY is primarily secreted by endocrine cells in the distal small bowel and colon <sup>122</sup>. Dipeptidyl peptidase-IV (DPP-IV) hydrolyses PYY and converts the precursor

PYY<sub>1-36</sub> to PYY<sub>3-36</sub>. PYY<sub>3-36</sub> is an appetite suppressant. In humans, infusions of PYY<sub>3-36</sub> comparable to those after a meal resulted in decreased energy intake at subsequent meals compared to a control group<sup>123</sup>. In this study, the effect was relatively long lasting (approximately 12 hours) and subjects did not report feeling more hungry. Although, PYY<sub>3-36</sub> is released lower in the bowel, increases in the peptide occur before foods reach the large bowel, so it would seem that a neural mechanism exists, most likely via the vagus nerve<sup>124</sup>. In addition to this initial effect, unabsorbed nutrients in the distal colon, as occurs with diets high in fibre, activate an ileal brake to slow gastrointestinal transit time. This mechanism is likely in part due to secretion of PYY<sub>3-36</sub><sup>125</sup>.

A landmark study in obesity research by Batterham et al<sup>126</sup> showed that obese individuals were not resistant to the anorectic effects of PYY<sub>3-36</sub>. Obese individuals tend to have lower endogenous PYY<sub>3-36</sub> so correction of this anomaly or its effects through pharmacological means became a legitimate pursuit for obesity researchers. Decreased endogenous PYY has also been demonstrated in obese children, with increasing levels after successful weight loss<sup>127</sup>. Variation in endogenous levels of PYY and its subtypes has not always been demonstrated<sup>122</sup>, but effects of PYY<sub>3-36</sub> in particular remain of interest due to its anorectic effects and variation of levels with weight loss.

Further research demonstrated that with infusion of exogenous PYY<sub>3-36</sub> a dose response relative to dietary intake exists<sup>128</sup>. Although this decrease in energy intake has not been recently supported, subjective increases in satiety and decreased hunger have been demonstrated<sup>129</sup>. PYY<sub>3-36</sub> infusion has also been shown to reduce plasma levels of ghrelin further adding to the appetite decreasing effects of PYY<sub>3-36</sub><sup>126</sup>. Hormones such as leptin, do not appear to regulate PYY at least in the short term, with fasting (2-3 days) induced low PYY, not altered by reintroduction of leptin in physiological or pharmacological doses<sup>130</sup>.

The release of PYY and PYY<sub>3-36</sub> in relation to food ingestion justifies its use in satiety research, but its altered levels in obesity make it of key interest.

### 2.2.1.3 Glucagon-like peptide-1

GLP-1 is synthesised by the same gut endocrine cells which produce PYY. It is synthesised as the large precursor preproglucagon and cleaved to active peptides such as glucagons, GLP-1 and glucagon-like peptide-2<sup>131</sup>. GLP-1 is released by the distal gut in response to food intake. When given intravenously, GLP-1 decreases spontaneous food intake. This is the case in experimental studies when GLP-1 is at high, and normal physiological levels<sup>132</sup>. Subjects receiving GLP-1 infusions feel less hungry or have decreased appetite. It is therefore likely that GLP-1 is important in the regulation of satiety.

Adam and Westerterp-Plantenga<sup>133</sup> found that GLP-1 was secreted at greater levels after meals containing increased fibre, compared to a control meal. Mechanisms of action could relate to decreased gastric emptying or the presence of nutrients in the large bowel. Of particular interest in the Adam study, the control group consisted of normal-weight subjects and obese subjects, and the GLP-1 release was lower in the obese subjects. This difference was not seen when the subjects were given the high fibre meal so most likely the effect is due to the fibre delaying digestion of nutrients.

GLP-1 also has a role in glucose homeostasis and therefore potentially a role in control or development of type-2 diabetes. GLP-1 increases the ability of pancreatic  $\beta$ -cells to respond to glucose, potentiating the effect of insulin. Incremental doses of glucose taken orally do not cause incremental insulin release, but rather, the action of GLP-1 keeps glucose levels relatively stable while similar (although slightly larger) amounts of insulin are released<sup>134</sup>. Infusion with GLP-1 whether centrally or peripherally causes insulin release and elevation of GLP-1 suppresses glucagon, further reducing plasma glucose. Importantly, research has shown that for individuals with type-2 diabetes, GLP-1 is much less effective in enhancing insulin secretion, so it is a logical pathway for intervention.

Antagonists to GLP-1 increase food intake and body weight<sup>131</sup> and receptor agonists have improved glucose control in patients with type-2 diabetes as well as reducing body weight<sup>135</sup>. It is difficult to elucidate precise effects of GLP-1 in appetite, independent of these insulin effects but regardless of precise mechanisms, interventions successful in



achieving weight loss are highly beneficial. The fact that  $\beta$ -glucan tends to decrease insulin release may be in part due to increased GLP-1 and this link with weight control means its measurement in satiety and weight control research is important.

#### 2.2.1.4 Ghrelin in acute satiety and as an adiposity signal

Ghrelin is a twenty-eight amino acid peptide with a hydroxyl group on a specific serine residue which is acylated by *n*-octanoic acid <sup>136</sup>. Ghrelin is found in a variety of tissues but is secreted primarily in the stomach. It exists in an acylated and non-acylated form and it is the former which has been shown to possess endocrine activities in part through its binding to the growth hormone secretagogue receptor (GHS-R) type 1a <sup>137</sup>. Ghrelin stimulates secretion of growth hormone, food intake and subsequent body weight gain when administered peripherally or centrally <sup>137</sup>. In particular, there is a pre-meal rise in plasma ghrelin <sup>138</sup>, which then decreases on cessation of fasting. It seems that this post-meal decrease may correlate to energy intake.

Although it was predicted that ghrelin would be elevated in obese individuals, the opposite has been found. It is possible that ghrelin is down-regulated in a state of energy excess <sup>136</sup>. Ghrelin has been identified as adipogenic and potentially acting as a longer term moderator of body weight through its interaction with leptin <sup>139, 140</sup>. Therefore the idea of blocking ghrelin or decreasing circulation of ghrelin, has provided a target for research into drug treatments for obesity <sup>141, 142</sup>.

It appears ghrelin is secreted in variable amounts depending on the macronutrients ingested. Monteleone and colleagues <sup>143</sup> identified glucose, insulin, leptin and ghrelin responses to isocaloric high-carbohydrate and high-fat meals. Subjects also rated subjective feelings of hunger. The high carbohydrate meal initiated a greater decrease in ghrelin than the high fat meal and the former also had greater effect to reduce subjective hunger. The changes in ghrelin were associated with the changes in hunger ratings <sup>143</sup> but other studies showing ghrelin changes do not always identify the hunger changes <sup>144</sup>. In a study investigating isocaloric meals with varied protein, fat and carbohydrate <sup>145</sup>, high carbohydrate meals once again caused the greatest post-meal decrease in ghrelin, although the high protein meal saw the suppression maintained significantly longer (measured to 180 minutes post-meal).

The limited effect of fat compared to other nutrients on entry to the gut may relate to the lack of digestion within the stomach. Infusions of long chain fatty acids into the duodenum suppress circulating ghrelin but not in the presence of a lipase inhibitor <sup>146</sup>. So it seems that fat digestion is required for ghrelin suppression and other post-digestive mechanisms such as insulin secretion for carbohydrates and small intestine osmotic changes for proteins are relevant <sup>141</sup>. In particular, the study using duodenal infusion is interesting as the site of ghrelin production is essentially the stomach, and its production is altered even without nutrient contact with the stomach.

The range of responses of ghrelin to different nutrients is intriguing and as fibre has been traditionally considered a satiating ingredient and ghrelin the only appetite stimulating hormone, measurements will be included in the research in this thesis.

#### 2.2.1.5 Acute Hormone Summary

All of the hormones described, have their concentrations altered by food intake or change in response to food intake. Macronutrient levels effect the amount of hormones released, although all nutrients have some effects. Some hormones require gastric distension, for effect, whereas others are independent of this. Some hormones affect the levels of others and while the precise mechanism is not recognised there is no doubt that just as the aetiology of obesity is complex, using a single pathway for treatment of this condition may leave still others open to manipulation or to be over-ridden. In obesity research, measurement of as many hormones as possible in response to a treatment (food or pharmacological) will give the most precise picture of mechanism which will only enhance further research opportunities.

#### 2.2.1.6 Insulin

In mechanisms of energy homeostasis, insulin is considered a longer term regulator of body weight, although it fluctuates significantly with every meal. Fasting plasma insulin and insulin responses to a meal are both correlated with body adiposity <sup>147</sup>. The release of insulin is controlled by regulation of innervation of the pancreas, the effect of nutrients ingested, and the stimulation by hormones such as GLP-1. Insulin mechanism of action appears to relate to action as a negative feedback signal of recent energy intake and body adiposity <sup>147</sup>. High insulin may promote overweight and obesity by

enhancing appetite and altering adipose tissue physiology <sup>148</sup>. Recently, insulin has been implicated in hypothalamic control of blood glucose and energy homeostasis via various central nervous system receptors and pathways <sup>149</sup>.

Glucose is the exclusive energy source of the brain, and has limited variation in healthy individuals. Decreasing glucose prior to a meal has been postulated as a specific cue for meal initiation but with limited links to satiety <sup>116</sup>. Insulin action to control blood glucose means it is not easy to use it as an independent marker of satiety. That is, acute levels correspond to the food ingested, especially carbohydrate. So although endogenous levels of insulin correlate with adiposity, infusions of exogenous insulin do not correlate with appetite or energy intake <sup>116</sup>.

A more recent meta-analysis of test meal studies showed post-prandial insulin association with appetite regulation in normal weight subjects, but satiety was not necessarily increased in the overweight/obese population <sup>150</sup>. Central nervous system pathways in insulin resistance as described by Morton (2007) or via interactions with CCK, GLP-1 or PYY may explain such effects and measurement of insulin in conjunction with these hormones may be important in satiety research. In addition, as insulin is a useful marker of adiposity, review of changes in insulin are useful in chronic studies of weight change.

#### 2.2.1.7 Leptin

Leptin is released mainly by adipose tissue and provides feedback for food regulation and energy balance via the central nervous system. Leptin levels do not change acutely in response to meals, although when energy deficit <sup>151</sup> or surplus <sup>152</sup> last for greater than twenty-four hours plasma leptin is strongly negatively correlated with appetite and food intake. Change in leptin during longer term negative energy balance (2-4 days) is considered a biomarker of satiety <sup>116</sup>. Therefore in studies of patients on energy deficient diets (or hypercaloric diets), leptin is a useful measure. Leptin changes after weight loss and weight gain have also been linked to difficulties in individuals to sustain weight loss or gain due to adaptive mechanisms initiated in the hypothalamus by leptin <sup>153</sup>.

Leptin activates hypothalamic pathways in glucose homeostasis which may be independent of its action on food intake <sup>149</sup>. Insulin resistance and glucose intolerance, which are common in obesity, may be a consequence of impaired or down-regulated leptin pathways and therefore a target for treatment.

### 2.2.2 Subjective measures of satiety

Appetite has many facets and cannot be directly measured. Examination of appetite includes measuring hunger, satiety and satiation. Although the biochemical measures described above contribute to the knowledge base, other somewhat subjective methods of measurement must also be used. This includes use of VAS and other questionnaires, and assessment of food intake patterns and quantity of intake.

The linking of food intake data to appetite is difficult as lack of availability of food (for financial reasons, or due to poor palatability or temporarily decreased access or illness) or intake in excess of appetite such as in disordered eating or even “stress” eating, will effect the outcome of any such measurement <sup>154</sup>. In addition to this, measuring food intake outside laboratory conditions is complex and fraught with difficulties yet within the experimental setting, other difficulties exist. Individuals may falsely represent usual intake or researchers may create artificial environments which affect appetite <sup>154</sup>.

Within these limitations, the use of VAS to assess acute satiety is commonplace. Typically, subjects rate desire to eat, hunger, fullness and prospective food consumption (need to eat a large amount or none at all) on a linear 100mm scale. Subjects simply mark the point which represents how they feel at that time. These scales can be used for several days at regular time intervals in longer term studies, or most typically, they are used for one to six hours after a pre-load meal to record sensations before and after the food intake. Often the level of *ad libitum* consumption of other foods after the test meal is also measured. The range of questions typically used, are listed in Figure 2.1.

**Figure 2-1 Visual analogue scale questions**

Questions included in visual analogue scales to measure satiety
How hungry are you right now? How strong is your desire to eat right now? How much could you eat right now? How full are you right now? How strong is your desire to consume something sweet right now? How strong is your desire to consume something savoury right now? How thirsty are you right now?

The ability of these questions to accurately assess appetite and especially subsequent food intake has been questioned <sup>154</sup>. Significantly, studies have examined dietary intake against the appetite ratings with mixed results. Doucet et al <sup>155</sup> showed no consistent association between appetite ratings after a meal and daily energy intake. This study utilised obese patients before and after some weight loss and the disease aetiology of obesity may play a part in the negative result. Parker et al <sup>156</sup>, reviewed four single-blind, randomised, controlled appetite studies to show food intake was related to perceptions of hunger and fullness in healthy adults of “normal” weight.

In a study designed to specifically measure reproducibility, power and validity of VAS in single test meal studies, Flint et al <sup>157</sup>, found that the strongest correlation to energy intake, was the 4.5 hour mean VAS scores for the appetite ratings (questions 1-4 above). The 4.5 hour scores were the final time-point for these subjects before they could consume *ad libitum* from a buffet meal. Diet standardisation prior to the test day did not influence results. An effect of 10% change in appetite can be considered reasonable. Flint’s work showed this change is likely to be detected if there are at least 18 subjects comparing fasting to mean appetite ratings with a power of 0.8. Results are most reproducible within individuals and so paired, repeated measures analysis can reduce numbers significantly.

A review of VAS has also noted that reliability and validity is more pronounced under the controlled laboratory conditions <sup>158</sup>. The review recognises that VAS are best used in conjunction with biochemical markers rather than as proxies for these markers.

Attempts to improve the standard VAS have summarised phrases related to hunger and fullness and assigned rankings based on group responses. These phrases were then

placed on a vertical line according to the mean magnitude of fullness/hunger determined by the group. This created a labeled magnitude scale of satiety<sup>159</sup> which compared well to VAS for sensitivity and reliability. Other attempts at improvement include the use of hand-held computerised systems for measurement of appetite (electronic VAS). These may be useful to record time accurately and stop participants completing ratings at incorrect times, such as recording at night for the entire day. However, there are differences in the techniques of pen and paper scales compared to the electronic versions so they should not be used interchangeably<sup>158, 160</sup>.

VAS have been used to develop a satiety index<sup>54</sup> and a satiety quotient<sup>161</sup>. The satiety index measures the ability of a food to satisfy hunger. Fixed energy intakes of different foods are given to participants who rank feelings of hunger on VAS and are allowed to eat freely for the following two hours. The intakes are recorded. The ranking is given as a figure in relation to white bread, which is arbitrarily assigned the value of 100. Given many episodes of eating are more than two hours apart, a criticism may be that many foods are satisfying within this time period. The satiety quotient rates the return of motivation to eat after the pre-load meal being measured. Results from VAS (using area under the curve data) rate motivation to eat before the meal and then after the meal. The difference is then divided by the weight or energy content of the meal to get an estimate of subjective satiety.

### 2.2.3 Dietary Intake

Measurement of dietary intake is the gold standard in appetite research as it is clearly linked to weight control, which is the primary long term goal of most appetite research. Where biochemical markers assist in determinations of mechanisms and subjective measures quantify subjective feelings or intent to consume, ultimately appetite is studied to assist in helping individuals consume more or less. At some point the amount an individual consumes must be quantified in relation to any intervention.

Dietary intake can be described as a summary of the episodes of eating of an individual. Increased satiety at a single time point does not necessarily mean a decrease in total energy intake as intake can simply increase at a later meal. It is argued that individuals and their food intake in an environment of surplus is a complex array of environmental,

psychological and physiological factors <sup>154</sup>. Given that the study of appetite is mainly pursued to control obesity, eating disorders or other serious illness, the link between appetite and “disease” must continue to be pursued. That is, in study of obesity, it must always be confirmed that decreasing appetite will result in overall decreased energy intake and subsequent weight loss. Long term studies are required, not just acute feeding studies.

It is difficult to measure how long effects from an individual food item will last, or if there would be compensatory mechanisms at other times in the day. Animal research identifies that a protein leverage hypothesis describes locust behaviour. That is, locusts will continue to eat until they gain enough protein from their food source, which can result in over- or under-consumption of kilojoules <sup>162</sup>. It would be far more difficult to show such a process in humans given the much greater subjectivity of both consumers and researchers and the far greater variety in food supply. However, research relating to bodily mechanisms to regain weight when lost and regulation of metabolism is important. A set-point theory of body weight control is still controversial <sup>163-165</sup>, but undoubtedly of some relevance in weight control research. Even still, decreasing food intake over time will result in weight loss, so dietary intake still serves as both a reasonable proxy for weight loss but also a tool for quantifying compliance and energy expenditure.

Dietary intake in a research setting is typically recorded by direct observation, (including weighing of food before intake and review of food remaining), dietary recall (of typically 24 hours), diet history taking (by a qualified professional) or food records (completed by the subject). Depending on the type of study, one of more of these techniques is used. To measure acute effects of a food or ingredient, direct observation is usually used in meal test studies. The intake before this meal is quantified most often by record or recall, and any meals after the first are often recorded by the patient rather than the researcher.

Recording weighed food intake in a laboratory setting is accurate, but the artificial environment may alter outcomes. For example, more food may be eaten as the food is prepared for subjects and often offered in excess quantities or less food may be eaten if the subject is conscious of the observation of intake. The psychological factors

involved in eating behaviour must always be considered. Individuals do not just eat when they are hungry and their intake will vary depending on social or clinical situations. In particular, overweight or obese subjects may have specified dietary habits based on their previous dieting <sup>166</sup>. A recent study identified the reproducibility of ad libitum intake as a measure of spontaneous energy intake in meal test studies with or without prior meal test standardisation <sup>167</sup> and it would seem a valid measure in appetite research.

All other methods of recording, involving a full day of dietary intake, can be tested for accuracy using techniques such as Goldberg cut-offs which examine reported energy intake <sup>168</sup>. However, the existence of under- and occasionally over-reporting must still be recognised as a factor in dietary analysis. Methods to minimise inaccuracies, such as financial incentives <sup>169</sup> appear to make little difference. Similarly, attempts to categorise individuals most likely to alter records or under- or over-eat in general are not considered failsafe <sup>170, 171</sup>

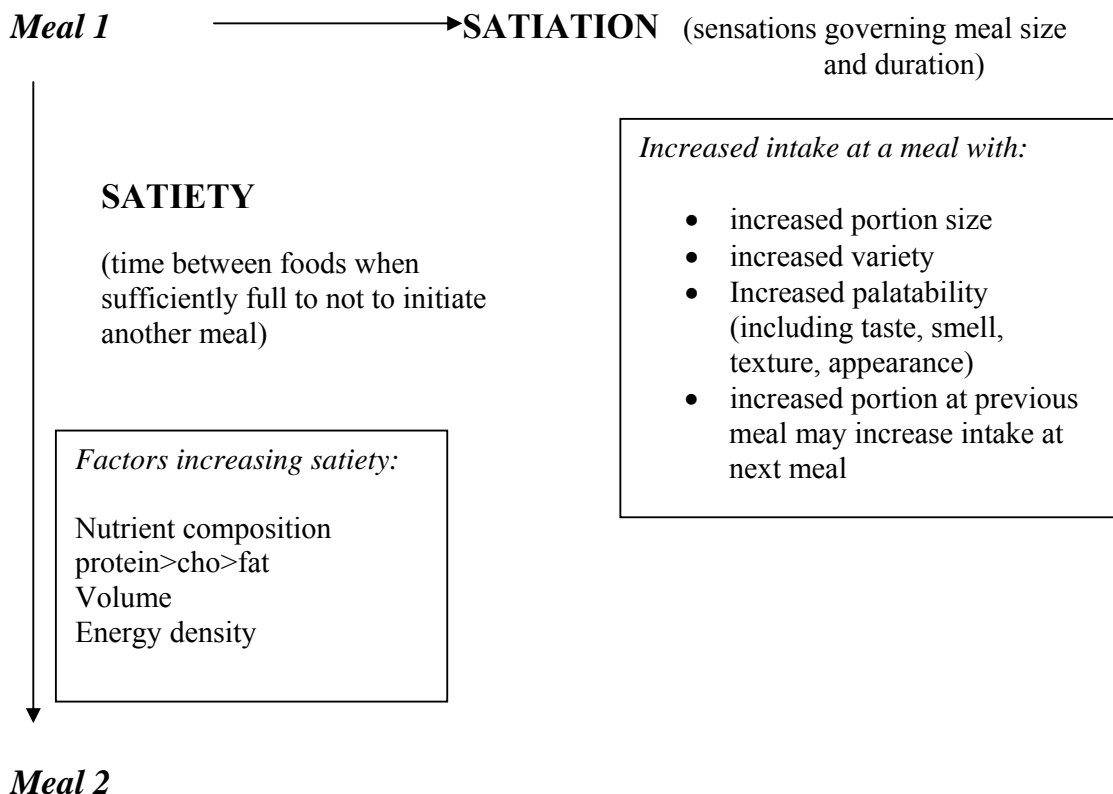
These measures include the Stunkard and Messick questionnaire designed to measure dietary restraint, disinhibition and hunger <sup>172</sup>. The three-factor eating questionnaire (TFEQ) as it is referred to, measures how individuals may restrict food intake to lose weight or prevent weight gain, how hungry they feel and how they may overeat as a consequence of emotional stimulus. Use of the TFEQ is helpful in dietary studies to categorise individuals in case of confounding results due to emotional or cognitive restraint or overeating, rather than due to the test variable. However, once again it is a useful tool, rather than a measure that should alter test protocols.

### **2.3 Food variables affecting satiety and satiation**

Figure 2.2 details the myriad of food factors shown to affect satiety and satiation. It is still important to recognise that these factors are often tested in individual meal-test studies and so it can not necessarily be translated to an overall increase or decrease in energy intake if these factors are altered. Organoleptic properties of foods offered in research and in real life are important – particularly as the study of appetite has been most promoted by obesity researchers and therefore food supply is abundant or perhaps “over-abundant”. In addition the volume of food and nutrient density are important



along with meals eaten previously. Great variation in these factors among test foods confirms the need for short-term feeding trials to be validated in longer term dietary trials. Meal test studies must control for all of these factors to gather a true measure of satiety in relation to the test ingredient.



**Figure 2-2 Factors affecting satiety and satiation** <sup>154, 173-176</sup>

## 2.4 Meal Test Studies

While there are significant considerations in interpreting data related to meal test studies, careful standardisation of protocols, controlling for background diet in the test and accurate data collection will maximise outcomes in a repeated measures design. Levels of hormones or food intake can only be interpreted in the experimental situation with relative measures compared under the same conditions. The studies are most useful when a mixture of outcomes are used, including VAS and ad libitum energy

intake and there is no need for prior diet standardisation when the outcomes relate to these measures <sup>167</sup>. Importantly, meal test studies will only define the acute effects of a particular food. Levels of food intake after ingestion of a test meal are a good indication of satiety effects. Similarly high levels of anorexigenic hormones indicate that a subject is likely to feel satiated. However, hormone levels do not always correlate to subjective satiety <sup>177</sup> or dietary intake <sup>178</sup> and hormones levels are a guide to mechanisms, that is a marker, rather than a true outcome measure of satiety. Meal test studies used in this thesis consider these caveats.

## **2.5 Randomised Controlled Trials**

The Australian peak clinical research body, the National Health and Medical Research Council have guidelines for assessment and application of scientific evidence. This document identifies evidence from a systematic review of all relevant randomised controlled trials (RCT) as the highest level of evidence, with evidence from at least one properly designed RCT as the second level of evidence <sup>179</sup>. This same standard applies to health claim substantiation by FSANZ. In investigation of a food component's effects on weight control, such a trial is therefore the obvious choice. However, there are still a number of difficulties inherent to any intervention trial.

Difficulties in dietary interventions include the inability of the researcher to be fully blinded to intervention and the control and measurement of the background diets. Unlike medication, changing one part of a diet affects other foods which may usually consumed and therefore control treatments actually require some degree of dietary intervention also. This is acceptable, but rigour in dietary recording, design and analysis is essential in interpretation of the results. Dietary analysis of all dietary intake before, during and possibly after a trial is essential. Common to all RCTs are the ethical issues associated with exposing patients to potentially inferior treatments <sup>180</sup> and the cost associated with measurements, interventions, product provision and support of patients within the trial. In addition, interpretation of results from a trial compared to “real life” situations may be difficult. However a recent review indicates that participation in RCTs is associated with similar outcomes to receiving the same treatment outside RCTs <sup>181</sup>. Recognition of these limitations assists in maximising the

integrity of the data and relevance to real life situations, a major consideration for this thesis.

## 2.6 Hypothesis

The general hypothesis addressed in the experimental components of this thesis is that overweight individuals following a nutritionally-balanced, energy-restricted diet including oat  $\beta$ -glucan will experience increased satiety and lose more weight than if they followed the same diet without the added  $\beta$ -glucan.

This hypothesis required substantiation of the satiety effects of  $\beta$ -glucan together with the outcome measure of weight loss to examine the possibility of a health claim related to  $\beta$ -glucan, satiety and weight control. Acute satiety, together with longer term benefits must be evidenced, with markers of satiety such as certain appetite hormones useful to inform mechanisms of action.

The specific aims of the original research included in this thesis were to:

1. Develop food products containing physiologically active  $\beta$ -glucan at a dose likely to elicit effects on satiety and longer term weight control.
2. Determine the effective dose of  $\beta$ -glucan to improve satiety in an acute setting.
3. Test the effectiveness of  $\beta$ -glucan from oats to increase satiety, when consumed as part of an energy-controlled diet.
4. Identify any differences in markers of satiety and weight control which are enhanced by  $\beta$ -glucan when consumed as part of an energy-controlled diet.
5. Provide comment on the scientific evidence for health claims related to satiety and weight control for commercial products high in  $\beta$ -glucan.

In order to address each of the research aims, three separate studies<sup>1</sup> were conducted:

<sup>1</sup> In addition to these studies a fourth study with animals also took place within our laboratory in association with the ARC-Linkage grant to determine possible mechanisms of  $\beta$ -glucan action and the dose effective in improving overweight and obesity in mice. The results and outcomes do not form part of this thesis, but provided supportive information for the experimental work contained in this thesis.

Study 1 – Development and testing of products to determine the choice of  $\beta$ -glucan source, together with testing of MW, solubility and viscosity throughout the product development phase to maximise the likelihood of producing products with physiological functionality.

Study 2 – An acute satiety meal-test study to test the effective dose of  $\beta$ -glucan in humans and measure acute satiety markers.

Study 3 - A 3-month randomised control clinical trial to test the ability of the developed products to enhance satiety and weight loss, with measurement of markers of these outcomes.

## **2.7 Study Design**

### **2.7.1 Development of $\beta$ -glucan enriched ready-to-eat cereal**

The literature suggests that a doses of approximately 4 g of  $\beta$ -glucan would show significant reduction in glycaemic response, and it is suggested at least this dose for other functions such as satiety and weight control <sup>71</sup>. Current marketed products containing  $\beta$ -glucan contain as little as 0.75 g  $\beta$ -glucan as this is the minimum 25% of the 3g dose specified by the US FDA for products to list a claim for a good source of  $\beta$ -glucan. These products avoid complications of very high concentration (which may decrease viscosity) <sup>112</sup> and are easy to ensure palatability - large quantities of fibre in a product may decrease palatability.

Given the importance of MW, solubility and viscosity in functionality, there were significant product development challenges. The final product made for the clinical trial and ultimately for a marketed product had to be palatable, contain physiologically significant quantity of  $\beta$ -glucan and be commercially viable. Uncle Toby's Propriety Limited as part of Cereal Partners Worldwide and Nestle, was the industry partner in the Australian Research Council Linkage Grant examining the role of  $\beta$ -glucan in weight control which funded research in this thesis. In-house food technologists took part in product development discussed in this chapter.

The following steps were included in the preparation for research and development detailed in Chapter 3 of this thesis.

- Investigation of  $\beta$ -glucan sources available within Australia and overseas together with agencies to test MW, viscosity and solubility.
- Production of a ready-to-eat (RTE) product under harsh conditions of temperature and extrusion. A cold cereal was chosen as this presented the greatest production challenge. Hot cereals only require inclusion of the additional fibre within the sachets and cereal bars are produced under less harsh conditions than cold cereal or are sometimes produced from cold cereals. All products were tested for MW, solubility and viscosity of  $\beta$ -glucan.
- Products with high viscosity, solubility and MW then defined the maximum temperature and extrusion conditions which may maintain the physiological function of  $\beta$ -glucan in a finished product. This product was then improved until it was organoleptically acceptable for use in the acute meal test (Chapter 4). This included development at varying doses to use in the acute meal test study to identify ideal doses of  $\beta$ -glucan for inclusion in longer term weight reduction interventions.

### 2.7.2 Meal-test study to measure acute satiety

A meal-test study was designed to test the levels of  $\beta$ -glucan which may provide acute satiety and therefore be of use in a longer term clinical trial. Subjective satiety using VAS, biochemical markers and food intake data were all utilised as outcome measures.

The study aimed to prove that foods containing increasing levels of the soluble fibre,  $\beta$ -glucan, are more effective in improving acute satiety (with the logical follow-on being that  $\beta$ -glucan is a useful ingredient in weight management diets). Overweight subjects were provided with four different test-meals ranging from negligible to high levels of  $\beta$ -glucan, on four separate occasions and a fifth control meal on a separate day. Appetite and satiety signals were monitored until the next meal, where ad libitum food intake was recorded. It was hypothesised that meals with higher levels of  $\beta$ -glucan would result in increased satiety. The study was a within-subject repeated measures design with subjects tested at the same time of day under as similar conditions as possible.

Primary outcomes were differences between responses to meals with respect to

- energy intake at the subsequent meal
- VAS scores (satiety)
- biochemical markers of appetite – CCK and ghrelin

Secondary outcomes included glycaemic and insulinaemic responses to the meal.

Cereal Partners Worldwide and Nestle funded this project as a fee for service project.

Subsequently to the original data analysis within this study and with results from animal studies within our laboratory available, spare duplicate samples were tested for total PYY. PYY served an additional biochemical marker associated with satiety to identify responses of different doses of  $\beta$ -glucan.

### 2.7.3 Satiety and weight control – 3 month human clinical trial

This study aimed to prove that foods containing  $\beta$ -glucan would improve weight loss outcomes as part of an energy-restricted dietary regimen. This would be at least in part due to the satiety properties of soluble fibre (notably increased  $\beta$ -glucan levels) which may improve satiation and satiety and therefore be useful in aiding dietary compliance.

Three groups of overweight subjects were provided with dietary advice on weight control diets and provided with supplies of unidentifiable test foods at varying  $\beta$ -glucan doses or their related control counterparts. It was hypothesised, that the diets high in  $\beta$ -glucan would result in increased satiety, improved compliance and hence greater weight loss, compared to the control group.

A 3-month parallel-randomised controlled trial was conducted in three groups:

Group 1: Low-energy diet: dietary advice based on usual diet with an energy deficit of approximately 2MJ/day, including high fibre.

Groups 2 & 3: Low-energy diet +  $\beta$ -glucan: dietary advice based on usual diet with an energy deficit of 2 MJ/day, including high fibre, but with two different but additional doses of  $\beta$ -glucan (determined in the acute study).

Satiety was assessed using VAS at various time-points. Body weight and percent body fat were measured, in addition to waist circumference. The following blood measurements were also investigated: blood glucose, cholesterol, triglycerides, HDL, LDL, leptin, ghrelin, insulin, CCK, GLP and PYY and PYY<sub>3-36</sub> all at 0 and 3 months.

The primary outcome measures were differences between groups for changes in body weight, percent body fat and satiety.

Secondary outcomes included:

- Differences between groups for changes in energy intake.
- Differences between groups for changes in fasting leptin, insulin and glucose levels, as well as CCK, ghrelin, GLP and PYY.
- Differences between groups for changes in fasting cholesterol, HDL and LDL.

The project was funded under an ARC Linkage Project ID number LP0561586 (Chief Investigators Huang, Tapsell and Batterham).

In light of the recently proposed changes in general level claim requirements related to fibre (P293 awaiting acceptance), which elevate the grams required for a “source of fibre” and a “good source of fibre” to greater levels, an analysis of the diets of subjects in relation to these categorisations was also included as Chapter 6 of this thesis.

## **2.8 Outcome Measurements**

The outcome measurements for the human studies within this thesis are summarised below in Tables 2.1.

**Table 2-1 Outcome measurements in acute and long term studies**

<i>Target Functions</i>	<i>Study type</i>	<i>Outcome Measure</i>
Reduces the risk of body weight gains	3 month trial	insulin leptin
Contributes to body weight reduction	3 month trial	weight loss
Decreases body fat	3 month trial	bioelectrical impedance, leptin, insulin
Reduces abdominal fat	3 month trial	waist circumference
Helps reduce energy/food intake	Meal test study	Compare ad libitum food intake between groups
	3 month trial	Compare energy intake between groups
Reduces appetite Increases satiety	Meal test study	VAS, ghrelin, CCK, PYY
	3 month trial	VAS, ghrelin, CCK GLP-1, PYY, PYY3-36

## 2.9 Significance of research

The research in this thesis makes a contribution to establishing efficacy, together with the development of an organoleptically acceptable product containing  $\beta$ -glucan by the commercial partner. The scientific hypotheses addressed by each of the described studies were that

1. the  $\beta$ -glucan within a prototype product was of high molecular weight, had a high viscosity and was soluble within the aqueous environment of the GIT.
2. the amount of  $\beta$ -glucan provided improved both subjective measures of satiety and biochemical markers of satiety.
3. overweight individuals who consumed high levels of  $\beta$ -glucan as part of a high fibre, energy-controlled diet, would lose more weight than individuals following a regular high fibre, energy-controlled diet.

Results from these studies were reviewed together with available literature to compile evidence related to  $\beta$ -glucan and satiety and/or weight management within a scientific framework for substantiation of health claims on food, informed by the PASSCLAIM project and the current Australian regulatory standards.



### **CHAPTER 3      DEVELOPMENT OF $\beta$ -GLUCAN ENRICHED CEREALS**

The experimental work in this chapter was carried out in conjunction with the Industry Partner of the ARC Linkage Grant – Cereal Partners Worldwide with use of a pilot processing facility. Determinations of physical characteristics of these products took place in Canada at Agriculture and Agri-Food Canada.

### 3.1 Introduction

The physiological action of  $\beta$ -glucan is a function of molecular weight (MW), solubility and final viscosity. Physiological functions of  $\beta$ -glucan include reduced glycaemic index, ability to decrease cholesterol and potentially an effect to improve satiety. The viscosity of a sample is a function of the MW and the concentration in solution, so any changes in parameters such as MW could affect physiological response. Studies have demonstrated poor results when  $\beta$ -glucan has been damaged by depolymerisation such as by  $\beta$ -glucanases in breadmaking (MW of  $\beta$ -glucan is decreased)<sup>107</sup>. The glycaemic response elicited by a test food is correlated to viscosity<sup>112</sup>, and lack of cholesterol response to high dose  $\beta$ -glucan is often attributed to variability in processing<sup>107</sup>. Other processes such as freezing high  $\beta$ -glucan products attenuates hypoglycaemic responses by individuals<sup>111</sup>, so it is likely that specified production and storage conditions are required for all  $\beta$ -glucan products to ensure MW, viscosity and solubility. For products made through extrusion,  $\beta$ -glucan could be affected by temperature or shearing during processing both of which could decrease the MW, although these processes have been less frequently reviewed.

Extrusion is a common food processing and manufacturing method, especially used in Ready-To-Eat (RTE) cereal production. Twin screws are intermeshing and co-rotate or counter-rotate to push ingredients through a machine (the extruder) at varying temperatures and pressures. Final conditions depend on the original composition of the materials, the flow rate of ingredients, the temperatures at which the machine is set, the shear rate and the pressure created. Creating a matrix of conditions (primarily altering temperature and shear rate), and measuring viscosity, solubility and MW of the final samples will define the maximum or most severe conditions under which a product containing  $\beta$ -glucan can be processed and still provide potential physiological benefits which may require varied viscosities.

From a manufacturer's perspective, it is critical to determine a minimal dose of a high cost ingredient which will still deliver the physiological benefits to maximise commercial benefits of a product. In research, products are initially tested at high dose to identify any possible effects. For example,  $\beta$ -glucan has been tested frequently at

greater than 5g doses<sup>65, 66, 92</sup>. Consumption of “foods” (such as oats) at this level of fibre is not acceptable as a regular eating pattern or at least difficult, due primarily to the volume and bulk required. Therefore, product development of concentrated oat bran products is suggested<sup>71</sup>. There are additional costs associated with functional ingredients and so minimising the dose of this higher cost ingredient is a goal for manufacturers. In a “pilot” phase of manufacturing as described in this chapter, varying doses will be developed for testing of physiological effects in acute meal test studies. In the case of  $\beta$ -glucan and its actions on satiety, the dose response is not well investigated. This contrasts with the cholesterol lowering dose responsiveness which is well documented<sup>76, 80, 84</sup>.

In addition, manufacturers need products which are marketable – both likely to attract a health claim relating to physiological benefits, but also meeting consumers demands for quality, including texture and taste. Even within a research framework, investigating the possible health benefits of  $\beta$ -glucan in a longer term feeding trial, an organoleptically acceptable product is required to ensure compliance with a dietary regimen. Products can be trialed in an acute study, where only a single consumption is required, for evaluation for longer term interventions.

Finally an important consideration which may affect functionality of  $\beta$ -glucan is the source of  $\beta$ -glucan. Differences in concentrations exist in different types of oats, and different methods of concentration or extraction may likewise, produce different products. Differences may relate to the concentration, the solubility, the palatability and importantly the micro-environment within which the  $\beta$ -glucan is contained. Comparing different sources of  $\beta$ -glucan within a single trial will elucidate differences and similarities so that functional differences can be recognised, but also so that a full cost-benefit analysis can take place.

The aim of the current study was to determine the maximum temperature and maximum shear rate of extrusion which cereal high in  $\beta$ -glucan can be manufactured and still maintain high MW, solubility and viscosity, while remaining organoleptically acceptable for use in dietary trials. Cereals with differing  $\beta$ -glucan contents, with  $\beta$ -glucan sourced from different manufacturers were extruded at varying temperatures and at various shear rates. Molecular weight, viscosity and solubility of all samples were

then determined and compared in conjunction with Agriculture and Agri-Food Canada at their facilities in Canada.

### 3.2 Methods

For initial analysis, raw mix of cereal ingredients was prepared in the proportions detailed in Table 3.1. The ingredients were calculated to provide a high dose (4.5g minimum)  $\beta$ -glucan (HBG) in the final serve of a finished product. Two  $\beta$ -glucan sources – a 22%  $\beta$ -glucan oat bran and an approximately 50%  $\beta$ -glucan product produced by an extraction process were incorporated into cereals tested (HBGO and HBGX respectively). These samples as well as the raw  $\beta$ -glucan sources with only water added were tested for their general extrusion properties as well as varied conditions of temperature and shear rate. Cereals as described in Table 3.1 were then mixed and extruded. An APV MPF50 twin screw extruder (Baker Perkins Inc, Grand Rapids, MI) was used. Calculated nutritional compositions of the raw cereal mix are included in Table 3.2 and were designed to deliver the 4.5g dose of  $\beta$ -glucan in a 45g cereal serve.

**Table 3-1 Ingredients included in formulations for testing**

<i>Ingredient</i>	<i>Extracted <math>\beta</math>-glucan - HBGX (g/100g)</i>	<i>Oat Bran <math>\beta</math>- glucan – HBGO (g/100g)</i>
B-glucan source	20	45.5
Oat Flour	30	5
Maize Flour	35	35
Maltodextrin	5	5
Sugar	9	9
Salt	0.5	0.5
Bicarbonate	0.5	0.5

**Table 3-2 Calculated nutritional composition of cereal formulations**

<i>Nutrient</i>	<i>Extracted <math>\beta</math>-glucan – HBGX (g/100g)</i>	<i>Oat Bran <math>\beta</math>-glucan – HBGO (g/100g)</i>
Carbohydrate (g)	61.6	53.8
Protein (g)	6.7	12.1
Fat (g)	3.8	2.8
Total Fibre (g)	17.7	20.9
Beta-glucan (g)	10	10
Total Kilojoules	1478	1293

Temperature within the extruder is affected by the original viscosity of the mixture, the shear rate and the temperature at which the equipment is set. Therefore the aim was to extrude the samples at temperatures considered low, medium and high. However, the final temperatures prior to final emission from the extruder could not be pre-determined as the extruder conditions and cereal formulation may alter the shear rate. This means cooling to maintain these temperatures is not adequate and greater temperatures are achieved. In addition to each of these three starting temperatures combinations, three different shear rates were applied to the extruder, creating nine different conditions for each of the  $\beta$ -glucan sources. In order to quantify and verify the range and variation in conditions, specific mechanical energy (SME) was calculated for each condition, where SME is a function of screw speed, motor torque, motor power, and flow rate. Bulk density (g/L) was determined for each sample immediately after collection from the extruder. Samples were dried at 50 degrees Celsius (to ensure no further damage of  $\beta$ -glucan) overnight.

Final samples, produced as a “cereal bubble” type cereal were sent to Agriculture and Agri-Food Canada where measurements of viscosity, MW and solubility took place. The total  $\beta$ -glucan was measured by the method of Glennie-Holmes and McCleary<sup>182</sup> using a kit from Megazyme (Megazyme International, Bray, Ireland). The  $\beta$ -glucan was extracted at 37°C following the *in vitro* digestion protocol of Beer and colleagues<sup>183</sup>. The viscosity of the extract was determined using a controlled strain rheometer (TA Instruments, NJ) and apparent viscosity at 30<sup>-s</sup> was reported. For the extracted  $\beta$ -glucan ingredient, the  $\beta$ -glucan content was too high to allow 5g to be hydrated in 100 mL of

buffer. A 2g sample was used for this ingredient. The concentration of glucan was determined by flow injection analysis (FIA) following the method of Jørgensen<sup>184</sup>. MW of the  $\beta$ -glucan in the extract was determined by size exclusion high performance liquid chromatography<sup>185</sup> except that the columns were Shodex OHpak KB806M and Waters Ultrahydrogel (Waters, Milford, MA). Moisture was determined by drying a weighed sample in a vacuum oven at 80°C for 5 h and measuring the weight loss.

Final results of these temperature and shear rate trials were used to develop a range of cereals for an acute feeding trial (Chapter 4) which were then also tested for concentration, MW, viscosity and solubility using the above methods. These cereals varied in source and concentration of  $\beta$ -glucan, ranging from a low dose (approximately 2g) to high dose (approximately 5g). Two high dose cereals were developed, one sourced from the previously described oat bran ingredient and the other from the extracted  $\beta$ -glucan ingredient.

### **3.3 Results**

Overall, it was not difficult to produce a cereal which was organoleptically acceptable for an acute trial. All formulations were deemed acceptable when taste tested within the staff at the production facility. The primary negative criticism related to the product served with milk, which when left to stand, allowed the cereal bubbles to stick together. In the context of product development for individuals volunteering for acute feeding trials, this was not considered a problem. However, further product development would be required for commercial production. Cereal products are pictured in Figures 3.1 and 3.2.

**Figure 3-3 Varied cereal samples dried overnight**



**Figure 3-4 The final product deemed acceptable for use in an acute feeding trial**



Details of results from the extruder are described in Appendix 3-1, with stages describing the temperatures at various positions throughout the extruder.

The following tables represent the analysis and comparison of samples for viscosity, solubility and MW of  $\beta$ -glucan (Table 3.3 and 3.4) raw products and those at varying temperature and shear rates. Table 3.3 shows the high molecular weights of raw oats, even if processed to be more easily prepared for consumption (quick cook oats). Similarly the raw oat bran and extracted  $\beta$ -glucan ingredients show high molecular weight. Solubilities are all relatively high. Table 3.4 indicates the changes after extrusion, although all molecular weights are still considered high and likely to allow viscous entanglements in the GIT. Solubilities were improved by extrusion while viscosity was maintained to a slightly greater degree with lower temperatures. Table 3.5 shows results for the cereals developed for use in the acute trial (Chapter 4) with high molecular weights, good solubility and viscosity increasing as a function of concentration.

Viscosity graphs and determinations from Dr Susan Tosh (Agriculture and Agri-Food Canada) are detailed in Appendix 3-2.



**Table 3-3 Characterisation of  $\beta$ -glucan raw ingredients, cereal mixes prior to extrusion and raw oats**

<i>Description</i>	<i>Moisture</i>	<i>Total Beta-glucan<sup>(1)</sup></i>	<i>% Solubility</i>	<i>Viscosity of extract (mPa.s)</i>	<i>Molecular weight</i>
Raw Ingredient (extracted $\beta$ -glucan)	6.55%	52.02%	39.14%	532 <sup>(2)</sup>	1,512,750
Raw Ingredient (oat bran)	4.09%	21.03%	62.24%	766.0	1,689,000
HBGO raw material formulation	6.57%	12.05%	46.77%	n/d	1,368,000
HBGX raw material formulation	8.94%	12.48%	68.17%	n/d	1,516,000
Uncle Toby's traditional oats*	9.41%	4.90%	58.98%	2.5	1,948,000
Uncle Toby's quick cook oats*	9.26%	4.90%	46.12%	2.1	1,993,500

<sup>1</sup> dry weight basis; <sup>2</sup> beginning with 2% solution vs 5% for other samples

\* additional cereals tested for characteristics of  $\beta$ -glucan existing in ingredients – no added  $\beta$ -glucan.

**Table 3-4 Characterisation of  $\beta$ -glucan cereals developed to test varying temperatures and shear rates.**

<i>Description</i>	<i>Moisture</i>	<i>Total Beta-glucan</i>	<i>% Solubility</i>	<i>Viscosity of extract (mPa.s)</i>	<i>Molecular weight</i>
HBGO--low shear (162°C)	5.51%	11.13%	93.76%	77.7	1,459,500
HBGO--low shear (170°C)	5.47%	11.74%	61.35%	49.6	907,000
HBGO--low shear (185°C)	5.51%	11.91%	78.02%	64.7	1,149,500
HBGO--medium shear (173°C)	6.38%	12.98%	59.08%	70.5	1,213,500
HBGO--medium shear (176°C)	6.52%	13.22%	60.47%	56.2	918,000
HBGO--medium shear (187°C)	6.68%	13.02%	64.90%	44.4	1,205,000
HBGO--high shear (NR)	4.60%	11.81%	60.66%	60.8	1,092,000
HBGO--high shear (177°C)	4.90%	11.42%	65.42%	50.7	1,179,000
HBGO--high shear (184°C)	5.08%	11.66%	77.95%	59.9	1,026,000
HBGX--low shear (NR)	5.77%	11.93%	68.04%	50.7	1,340,000
HBGX--low shear (170°C)	5.21%	13.33%	58.69%	60.9	1,031,700
HBGX--low shear (173°C)	5.57%	12.89%	66.44%	47.1	1,059,000
HBGX--low shear (181°C)	5.66%	13.30%	65.99%	44.2	1,133,000
HBGX--medium shear (169°C)	5.08%	12.36%	71.66%	59.6	1,082,250
HBGX--medium shear (175°C)	5.24%	12.75%	67.47%	45.4	920,000
HBGX--medium shear (182°C)	5.45%	13.02%	66.77%	37.9	1,130,000
HBGX--high shear (170°C)	4.55%	11.93%	67.54%	59.5	1,246,000
HBGX--high shear (173°C)	4.68%	12.00%	65.63%	55.7	1,179,000
HBGX--high shear (178°C)	4.81%	12.33%	71.45%	40.9	1,167,500

NR = not recorded

**Table 3-5 Characterisation of  $\beta$ -glucan cereals developed for acute meal test study.**

<i>Description</i>	<i>Moisture</i>	<i>Total Beta-glucan<sup>(1)</sup></i>	<i>% Solubility</i>	<i>Viscosity of extract (mPa.s)</i>	<i>Molecular weight</i>
Oat Bran Low Dose	4.65%	5.04%	67.91%	5.8	1,681,000
Oat Bran Medium Dose	4.91%	8.92%	78.08%	32.0	1,378,000
HBGO	4.09%	12.62%	70.47%	76.6	1,213,000
HBGX	3.83%	13.05%	71.86%	84.8	1,222,000

<sup>1</sup> dry weight basis

### 3.4 Discussion

The key elements in product development and subsequent  $\beta$ -glucan analysis are MW which must remain high to allow entanglements of  $\beta$ -glucan to increase viscosity but also the solubility, which should be high to allow formation of a viscous bolus in an aqueous gut environment.

Generally, under all extrusion conditions in which the products were developed, the MW remained high ( $>1$  million) and the solubility was relatively high. The raw products and ingredients tested give insight into naturally occurring parameters. For example, the highest MW measurements were seen in raw oats which have experienced the least processing. Both high  $\beta$ -glucan ingredients maintained high MW, but the oat bran ingredient was noticeably more soluble. However, when the other ingredients were added to the  $\beta$ -glucan source and extruded, the solubility of the extracted  $\beta$ -glucan product (HBGX) increased, while that of the oat bran formulation (HBGO) decreased. The difference in MW was negligible.

Review of the physical properties of the cereals at varied temperature and varied shear rate showed a general improvement in solubility and maintenance of MW under all conditions. Slight variations existed with slightly better viscosity measurements in the HBGO products overall, although this would not just be predicted by the MW and solubility measurements. The lower temperature and lower shear HBGO product was highly soluble and showed high viscosity. It should be recognised that all the results were acceptable to make the products, although use of the lower temperatures and shear rates were used for the development of products for the trials within this thesis. Also, although a low temperature was used in the study reported here, it was not particularly low for cereal production, as the high viscosity of the raw ingredients increased the final extrusion temperature.

In summary this research found that extrusion does not decrease viscosity as a relatively high MW was maintained. Solubility was enhanced by extrusion and overall, extrusion was found to be an acceptable manufacturing method for cereals of high  $\beta$ -glucan content.

## CHAPTER 4 ACUTE MEAL TEST STUDY WITH CEREALS CONTAINING $\beta$ -GLUCAN

### PART 1 – Acute effects of $\beta$ -glucan enriched foods

The majority of this section is the substantive content of the accepted work: Beck, E.J., Tosh, S.M., Batterham, M.J., Tapsell, L.C. & Huang, X. F. (2009) Oat  $\beta$ -glucan increases postprandial cholecystokinin levels, decreases insulin response and extends subjective satiety in overweight subjects. **Molecular Nutrition and Food Research**, 53, 1343-1351.

Discussion relating to this choice of test meal was included in the poster presentation, Beck, E.J., Norman, J.A., Batterham, M.J., Huang, X-F. and Tapsell, L.C. (2007) Dietetic Practice in Appetite Research – acute meal test studies. **Nutrition and Dietetics**, 64; Suppl.1, S49-S51. (Poster presentation from conference)

The results relating to dietary recalls and baseline biochemical measures and VAS are reported in Beck, E.J. Tapsell, L.C., Huang, X-F. and Batterham, M.J. (2008) Relationships between 24 hour recalls and fasting measures of hunger and satiety - subjective and biochemical indices. **Nutrition and Dietetics**, 65; (S2) A11(Oral presentation from conference)

Discussion relating to the multiple measurements required for assessment of  $\beta$ -glucan products was discussed in, Beck, E.J., Tosh, S.M., Tapsell, L.C., Batterham, M.J. and Huang, X-F. (2008) Characterisation of a functional ingredient requires multiple assessments. **Cereal Foods World**, 54(3) Supplement, A21.

## 4.1 Introduction

Dietary studies usually identify benefits associated with fibre and/or whole grains and postulate mechanisms related to ingestion, digestion and hormones associated with feelings of satiety. Identification of the mechanism by which fibre induces satiety may help to establish theoretical positions for undertaking longer-term weight reduction intervention trials. For example, if it is proposed that short-term satiety from fibre influences overall daily food intake, studies must first investigate the role of particular fibres within an acute setting. This would take the form of an acute meal test, recording recognised biochemical (appetite hormones) and subjective (visual analogue scales) measures and markers of appetite, along with food intake data from a subsequent meal/s.

Oats contain the soluble fibre  $\beta$ -glucan. Soluble fibres, such as  $\beta$ -glucan, influence appetite by chemical and physical properties (particularly their bulking action), and increase viscosity in the gastrointestinal tract <sup>2</sup>.  $\beta$ -Glucan improves glucose and insulin control <sup>88</sup>, yet a higher insulinaemic response may increase feelings of fullness when subjects are given a set amount of carbohydrate <sup>186</sup>. Any reduction in appetite or intake after ingestion of  $\beta$ -glucan must occur in spite of a lowered insulin response.

Few studies have measured appetite hormones in relation to  $\beta$ -glucan consumption. However, fibre appears to prolong CCK elevation after a meal, which should result in prolonged satiety <sup>119</sup>. Other hormones such as ghrelin, stimulate food intake <sup>137</sup>. There is a pre-meal rise in ghrelin <sup>138</sup>, which then decreases on cessation of fasting. A food, which could limit elevation of ghrelin either absolutely or via delay of elevation, would be of interest to appetite and obesity investigators.

A recent study showed variation in levels of appetite hormones after consumption of drinks containing a high level of  $\beta$ -glucan (>10g) depending on the amount of depolymerisation of the  $\beta$ -glucan by added  $\beta$ -glucanases<sup>105</sup>. The lower viscosity drink, presumably due to its faster gastric motility caused greater release of anorexigenic hormones in the first three hours post-ingestion.

It is recognised that variability in processing of oats, oat fibre concentrates and products delivering the  $\beta$ -glucan dose alters key parameters, especially solubility and viscosity<sup>107</sup> which contribute to  $\beta$ -glucan functionality. These parameters have a key role to invoke the mechanism of cholesterol lowering by  $\beta$ -glucan, and therefore most likely the mechanisms of satiety. Any investigation of satiety effects of  $\beta$ -glucan must measure concentration, solubility, viscosity and molecular weight, to help define the parameters of the  $\beta$ -glucan products used producing the desired effects.

This study used an acute meal test to identify (i) acute satiety effects of  $\beta$ -glucan in extruded breakfast cereal foods (ii) the dose responsiveness of these effects and (iii) any differences which may exist in the acute effects of  $\beta$ -glucan concentrated by different processes. To ensure the integrity and clearly characterise the  $\beta$ -glucan used in the study, concentration, solubility, viscosity and MW within the test food was also measured.

The study compared the effects of equal-energy breakfasts with different levels of  $\beta$ -glucan derived from oats on biochemical markers of satiety, feelings of fullness and subsequent *ad-libitum* food intake. Ghrelin and cholecystokinin, together with glucose and insulin, were measured as biochemical markers and subjects rated fullness using visual analogue scales. The minimum dose chosen was approximately 2g of  $\beta$ -glucan to represent a commercially viable dose, with higher quantities also evaluated to examine dose response. Comparisons between oat  $\beta$ -glucan from oat bran or extracted by an ethanol extraction were made at the highest dose to identify differences in effectiveness at a dose most likely to be effective in promoting satiety.

## 4.2 Methods

### 4.2.1 Recruitment

Subjects were sought through paid advertisement in local newspapers, by flyer advertisement at local gymnasiums and eating venues at a tertiary education institution. Institutional emails and ‘word of mouth’ were also used. In addition, subjects who had volunteered for other dietary studies within our laboratory were approached where suitable. Inclusion criteria were male or female, 19-45 years of age, body mass index range from 25-31 kg/m<sup>2</sup>. Exclusion criteria were smoking, known food allergies and lack of general good health.

### 4.2.2 Timeframes

All subjects attended an initial interview to collect dietary information and then five sessions of approximately five hours duration (breakfast to lunch). Visits were not allowed on consecutive days. Female subjects were either tested at the follicular phase of their menstrual cycle (days five to 12 of the cycle), or between days five to 22 if on anovulatory, non-triphasic contraceptives. Overall, this meant that female subjects covered a minimum of three months of their cycle but male subjects completed testing within approximately five weeks. Subjects were instructed to fast for a minimum of 10 hours prior to presentation for study appointments.

### 4.2.3 Test foods

Subjects consumed five different breakfast meals (Table 4.1) on five occasions after the overnight fast. Meals consisted of a bowl of cereal served with 200 mL reduced-fat milk and a glass of water. The cereals were a corn-based control breakfast, three cereals with varying levels of  $\beta$ -glucan (low - LBG, mid-range - MBG and high - HBGO) sourced from a high  $\beta$ -glucan oat bran, and one cereal with an oat  $\beta$ -glucan concentrate produced by an ethanolic extraction process (HBGX). Cereals were extruded from oat flour, maize flour, sugar, maltodextrin, sodium bicarbonate, salt, water and the  $\beta$ -glucan ingredient. An APV-MPF50 twin screw extruder (Baker Perkins Inc, MI) was used. Available carbohydrate and protein were matched using glucose polymer (Poly-Joule®, Nutricia Australasia) and protein powder (Beneprotein®, Novartis, United States) respectively, dissolved in the milk.



$\beta$ -glucan compositional analysis on the test foods (Table 4.1) was performed by Agriculture and Agri-Food Canada. Total  $\beta$ -glucan was measured by the method of Glennie-Holmes and McCleary (McCleary and Glennie-Holmes, 1985) using a kit from Megazyme (Megazyme International, Ireland). The  $\beta$ -glucan was extracted at 37°C following an *in vitro* digestion protocol (Beer et al., 1997). Viscosity of the extract was determined using a controlled strain rheometer (TA Instruments, NJ) and apparent viscosity at 30 s<sup>-1</sup> was reported. For the HBGX ingredient, the  $\beta$ -glucan content was too high to allow 5g to be hydrated in 100 mL of buffer, so a 2g sample was used. The concentration of  $\beta$ -glucan was determined by flow injection analysis following the method of Jørgensen (Jørgensen, 1988). MW of  $\beta$ -glucan was determined by size exclusion high performance liquid chromatography (Wood et al, 1991) except that the columns were Shodex OHpak KB806M and Waters Ultrahydrogel (Waters, Milford, MA). Moisture was determined by drying a weighed sample in a vacuum oven at 80°C for 5 h and measuring weight loss.

**Table 4-1 Composition and analysis of test meals.**

<i>Composition variable</i>	<i>Control<sup>i</sup></i>	<i>LBG<sup>ii</sup></i>	<i>MBG<sup>ii</sup></i>	<i>HBGO<sup>ii</sup></i>	<i>HBGX<sup>ii</sup></i>
cereal (g)	39	45	45	45	45
carbohydrate polymer (g)	-	-	4	8	3
beneprotein™ (g)	3	2	1	-	3
$\beta$ -glucan (g/serving)	-	2.16	3.82	5.45	5.65
2% fat milk (mL)	200	200	200	200	200
total protein (g)	13.3	13.4	13.4	13.5	13.0
total fat (g)	3.2	3.6	3.8	4.0	3.6
available carbohydrate (g)	43.6	43.2	42.9	42.6	43.3
total fibre (g)	1.2	3.7	6.7	9.7	7.8
energy (kJ)	1080	1098	1106	1115	1157

i Ingredients: Corn (90%), sugar, barley malt extract, salt, vitamins and minerals

ii Ingredients: Maize flour,  $\beta$ -glucan source, maltodextrin, sugar, calcium carbonate, bicarbonate soda, salt. LBG = low  $\beta$ -glucan dose, MBG = mid  $\beta$ -glucan dose, HBGO = high  $\beta$ -glucan dose (all containing  $\beta$ -glucan from oat bran), HBGX = high  $\beta$ -glucan dose containing extracted  $\beta$ -glucan.

#### 4.2.4 Anthropometric and dietary assessments

All subjects had their height, weight and waist circumference recorded. Percentage body fat was also measured using Tanita Scales (Model No. UM-019). A diet history was performed to collect information on usual eating habits. Participants also completed a three-day weighed food record. Scales, together with measuring cups and spoons were supplied to participants. This information was used to design the test meal

to be similar to usual intakes as well as of acceptable taste preferences to subjects. Twenty-four hour dietary recalls were also collected for the day prior to each study visit.

#### 4.2.5 Clinical measurements

On arrival at the clinical laboratory, subjects had a cannula inserted into the forearm and initial fasting blood samples were collected. The subject then ate one of the five test breakfast meals within a ten minute period. Fifteen minutes after completion of this meal, further samples were taken. Blood samples were then collected at 30, 60, 120, 180 and 240 minutes from completion of the meal.

Sarstedt™ Monovette Blood Collection tubes were used to collect samples for glucose, insulin, ghrelin and CCK analysis. The S-Monovette™ Serum-Gel tubes were used to collect approximately 4mL of blood to be sent to an accredited pathology laboratory for glucose (glucose hexokinase method, Roche Diagnostics, Australia) and insulin (electrochem-luminescence method, Roche Diagnostics Australia) analysis. A further 7-8mL of blood was collected into another S-Monovette™ containing potassium EDTA (to achieve a concentration of 1/2-2mg EDTA/mL of blood after collection) and aprotinin equivalent to 0.6 trypsin inhibitor units (TIU) per mL of blood. (Aprotinin Solution, NZ, manufactured by Serologicals, sourced from Chemicon Australia: Activity 5-10 TIU/mL.) The blood samples containing aprotinin were then centrifuged at four degrees Celsius for 15 minutes at 1500xg. The plasma was collected and distributed in eppendorf tubes. Tubes with samples for ghrelin analysis included 100µL of 1mol/L hydrochloric acid per one mL plasma collected. Tubes for both ghrelin and CCK analysis were stored at minus 80 degrees Celsius until further analysis could be completed.

Ghrelin analysis used Linco Research™ enzyme linked immunosorbent assay (EZGAC-86K) for measurement of the active octanoyl-modified portion of ghrelin. Protocols were as per the standard described analysis using this kit. CCK analysis required extraction of the peptides prior to analysis using Phoenix Peptides™ radioimmunoassay (RK-069-04) for cholecystokinin octapeptide (CCK 26-33). The standard radioimmunoassay protocol was applied.

#### 4.2.6 Appetite and hunger assessments

At each of the described seven time-points for blood collection, the subject was also asked to complete a VAS related to appetite<sup>157</sup>. The current study used four questions only – How hungry do you feel? How satisfied do you feel? How full do you feel? How much do you think you can eat? Subjects recorded their feelings on individual forms and results along each line were measured in millimetres on a 100mm scale.

Subjects remained within the clinical laboratory while all tests were performed. Four hours after completion of the breakfast, subjects were escorted to a kitchen area where a buffet lunch was served. A cold buffet was chosen as this most closely mimicked the typical lunch meal consumed by participants. The lunch consisted of a variety of sandwiches and flatbreads (cut into bite-size pieces), dried fruit, nuts, yoghurt and juice to a total of approximately 7500kJ. The foods offered combined to make a meal of 50% carbohydrate, 20% protein and 30% fat. Foods were weighed or measured before commencement of the meal and at completion. Consumed grams of protein, fat and carbohydrate and total kilojoules were calculated for each subject on each visit.

#### 4.2.7 Statistical Analysis

Previous studies identified that to detect biochemical differences, as few as seven subjects of each sex may be sufficient<sup>119</sup>, while 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<sup>157</sup>. Using a repeated measures design would decrease this to as few as 8 subjects<sup>173</sup>. A target of 15-18 subjects was set for recruitment based on these studies. Results for blood analysis, VAS values and second-meal dietary intake were entered into SPSS for windows, Version 15.0 and trapezoidal area under the curve (AUC) where values were corrected for baseline but areas below the baseline were also subtracted (netAUC as described by Wolever)<sup>187</sup>. Data was analysed for individual and combined sexes as past studies have identified some differences between sexes<sup>119</sup>. AUC differences in glucose, insulin, CCK, ghrelin and VAS results between the breakfasts were identified using repeated measures analysis of variance (RMANOVA) with post-hoc Bonferroni adjustments. For meal intake values, RMANOVA of kilojoules consumed was performed. Student's t-test was used to make comparisons between test meals. Regression analysis analysed relationships between dose,

biochemical, subjective and meal intake data and 24-hour dietary recalls. Biochemical results corrected for baseline and peak values were reviewed. VAS data from individual questions as well as the sum of four questions (converted from a measure of fullness to hunger where necessary) were analysed using RMANOVA of AUC. Cronbach's alpha was used as a measure of internal consistency between the four questions.

## 4.3 Results

### 4.3.1 Subjects

A total of 41 subjects were screened, of which 17 were recruited. The BMI inclusion range was increased to  $36\text{kg/m}^2$  due to significant recruitment difficulties. Two subjects withdrew due to changing work commitments after the initial interview in which background dietary data was collected, but before any intervention commenced. A third subject completed only two of the planned five visits due to time constraints. Fourteen subjects (seven female and seven male), aged 29-45 years (mean 38.7 years) with a mean BMI of  $29.6 \pm 2.95\text{kg/m}^2$  (range 25.2-36.6) completed the study. Average waist circumference was  $76.0\text{cm} \pm 11.2$  with body fat  $34.7 \pm 6.0\%$ . Fasting glucose measurements ranged from 2.7-5.7mmol/L (mean  $4.2 \pm 0.9\text{mmol/L}$ ) and fasting insulin 0.4–24.4mmol/L (mean  $9.5 \pm 5.4\text{mmol/L}$ ). Two individuals demonstrated consistently elevated fasting insulin and overall hyperinsulinaemia within the study. Data related to glucose metabolism was then reviewed including and excluding these outlier subjects.

### 4.3.2 Analyses of test foods

(completed by Dr Susan Tosh, Agriculture and Agri-Food Canada)

Results for analyses of test foods for total  $\beta$ -glucan content, solubility, viscosity and MW are detailed in Table 4.2. There was a slight decrease in peak MW with increasing concentration of  $\beta$ -glucan. Size exclusion chromatography shows the shift in the distribution of MW as the  $\beta$ -glucan concentration was increased (Figure 4.1). As the dose was increased, the peak became narrower, resulting in a gradual shift of the peak. However, the MW of the  $\beta$ -glucan would still be considered high enough to ensure functionality ( $>1,000,000\text{g/mol}$ )<sup>114</sup>. To estimate the effect of  $\beta$ -glucan on luminal viscosity, an in vitro digestion protocol was used. The cereal was treated sequentially with amylase (pH 6.9), pepsin (at pH 2) and pancreatin (at pH 6.9) at 37°C. In the

HBGX cereal, solubility of  $\beta$ -glucan was much higher than in the ingredient (72% vs 39%) and comparable to the solubility of  $\beta$ -glucan in all cereals made with oat bran ingredient (68-78%). As expected, soluble  $\beta$ -glucan concentration correlated ( $R^2 = 0.95$ ) with viscosity of the extract. Concentration strongly influenced viscosity, with doubling of concentration resulting in a 15-fold increase in viscosity.

**Table 4-2 Physico-chemical characteristics of  $\beta$ -glucan in test meals**

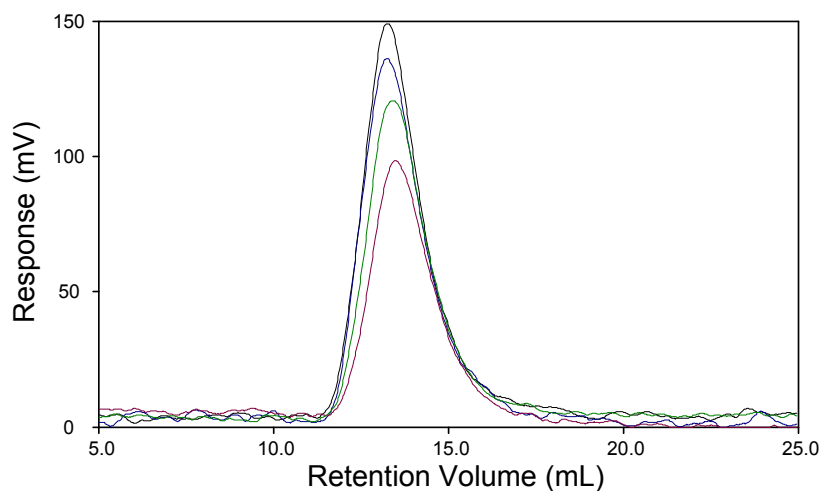
<i>Name of test food</i>	<i>Moisture (%)</i>	<i>Total <math>\beta</math>-glucan (% dwb)</i>	<i>Soluble <math>\beta</math>-glucan<sup>1</sup> (% dwb)</i>	<i>Viscosity of extract (mPa.s)</i>	<i>Molecular weight<sup>2</sup> (g/mol)</i>
Oat Bran*	4.09	21.03	13.09	766	1,689,000
Extracted $\beta$ -glucan*	6.55	52.02	20.36	532 <sup>3</sup>	1,513,000
LBG	4.65	5.04	3.42	5.8	1,681,000
MBG	4.91	8.92	6.96	32.0	1,378,000
HBGO	4.09	12.62	8.89	76.6	1,213,000
HBGX <sup>3</sup>	3.83	13.05	9.37	84.8	1,222,000

\*Raw ingredients

<sup>1</sup>Grams of extractable  $\beta$ -glucan per 100g of ingredient or cereal

<sup>2</sup>Peak molecular weight

<sup>3</sup>Viscosity of extract from 2g; other samples were 5g.



**Figure 4-1 Size exclusion chromatograms of  $\beta$ -glucan MW in the in vitro extracts.**

Peaks represent the distribution of  $\beta$ -glucan MW in, from top to bottom, oat bran (raw ingredient), LBG, MBG and HBGO. (Peak heights were adjusted to allow for better comparison and do not reflect concentration in the original extract.)

### 4.3.3 Baseline dietary intakes

Baseline dietary histories and food records indicated most individuals ate a cold lunch consisting of sandwiches or similar foods such as flatbreads or toast. When additional foodstuffs were consumed, this was most likely yoghurts, fruits, biscuits or other baked products. Hence, similar foods were chosen for the buffet-lunch meal.

Dietary recalls for the twenty-four hours prior to each visit averaged 9428kJ (3623-16845kJ). Female average intake was 7428kJ and for males 11,426kJ. As expected, regression analysis indicated some contribution of the food consumed in the previous 24 hour to the prediction of total lunch-time intake ( $R^2=0.132$ ,  $P=0.002$ ). However, no relationship was identified between the 24 hour dietary recall and the baseline measurements of any of the individual VAS questions or when the questions were combined to a single measure. Similarly, dietary recalls did not predict fasting insulin, glucose or ghrelin levels. A significant relationship ( $P=0.018$ ) was identified between 24 hour recall energy intake data and fasting CCK but review of the scatter plot showed this prediction was extremely weak ( $R^2=0.08$ ).

### 4.3.4 Clinical Indices

Insulin AUC data corrected for baseline indicated a significant difference between responses over the first two hours ( $P=0.011$ ) using RMANOVA (Table 4.3).

Regression analysis comparing soluble and total  $\beta$ -glucan to 2-hour AUC insulin, showed significant inverse relationships with  $R^2 = 0.95$  ( $P=0.005$ ) and  $R^2 = 0.97$  ( $P=0.007$ ) respectively. When the control meal was compared to each individual dose using a t-test, significant results were noted for the MBG ( $P=0.029$ ), HBGO ( $P=0.021$ ) and HBGX doses ( $P=0.014$ ). Removing results for subjects with hyperinsulinaemia showed improvement in insulin response for the LBG dose (Table 4.3).

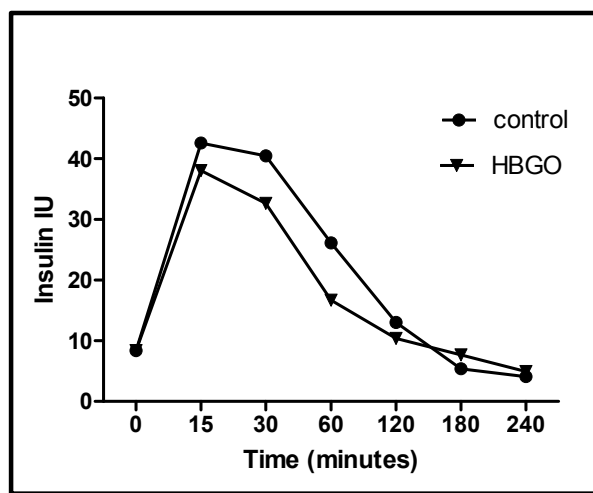
No significant differences were found with insulin reviewed over four hours (Table 4.3), although the same trend existed with decreasing insulin with increasing fibre. Raw data for post-prandial insulin responses for subjects (without hyperinsulinaemia) consuming the control and HBGO meals is depicted in Figure 4.2. No analyses showed any significant difference between the two HBG meals.

Raw data for post-prandial glucose responses is depicted in Figure 4.3 showing minimal differences between even the control and HBGO dose. There were no significant differences in glycaemic responses between test meals. This included analyses using RMANOVA of AUC data (Table 4.4) and comparison of peak glucose responses. Calculation of incremental area under the curve (method of Wolever et al <sup>188</sup>) also showed no statistical difference.

RMANOVA of AUC for ghrelin showed no significant results (Table 4.4).

RMANOVA of AUC for CCK trended towards a dose relationship ( $P=0.11$ ) (Figure 4.3). When data for each sex was analysed separately, this relationship was significant for women ( $P=0.048$ ) but not men ( $P=0.691$ ) (Figure 4.4 and Table 4.4). No significant differences were noted between HBGO and HBGX.

A dose response for CCK was noted (Table 4.4). Regression analysis for the dose of soluble or total  $\beta$ -glucan compared to the CCK responses identified significant relationships ( $R^2 \geq 0.97$ ,  $P=0.002$  for both). Student's t-test comparing the control meal to fibre doses identified results approaching significance for the combined sexes for MBG, HBGO and HBGX doses ( $P=0.062$ ,  $0.071$  and  $0.063$  respectively). When only female results are reviewed, the same doses produced significantly different results to the control ( $P=0.036$ ,  $0.032$ ,  $0.006$  respectively). No differences were identified between HBGO and HBGX for combined sexes or for women alone.



**Figure 4.2 Postprandial insulin responses for control and high  $\beta$ -glucan (>5g) cereal HBGO for subjects without obvious hyperinsulinaemia.**

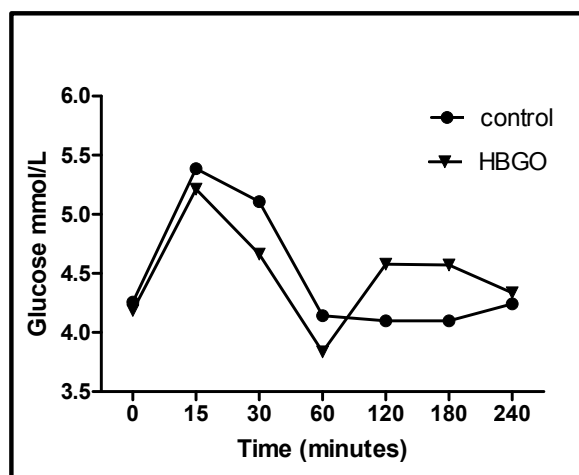


Figure 4.3 Postprandial glucose responses for control and high  $\beta$ -glucan (>5g) cereal HBGO.

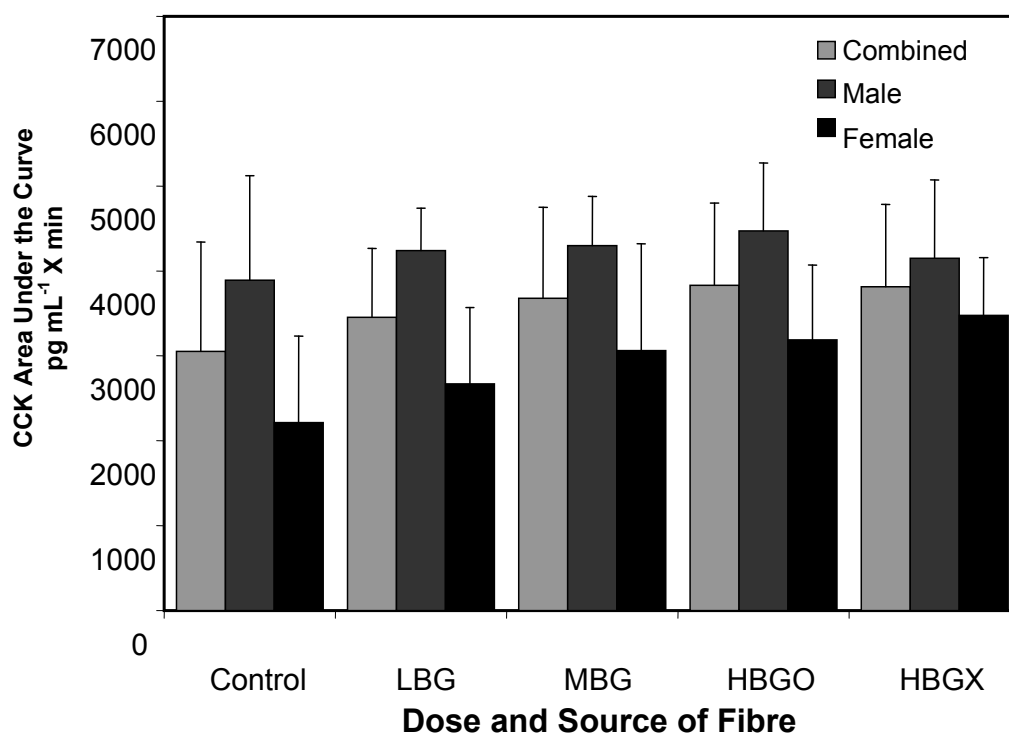


Figure 4.4 CCK response to various levels of  $\beta$ -glucan (combined sexes N=14; male N=7 and female, N=7)

LBG = low  $\beta$ -glucan dose, MBG = mid  $\beta$ -glucan dose, HBGO = high  $\beta$ -glucan dose (all containing high  $\beta$ -glucan oat bran), HBGX = high  $\beta$ -glucan dose containing extracted  $\beta$ -glucan.



**Table 4-3 Post prandial insulin (mU L<sup>-1</sup> X min) responses for 2 and 4 hours for all subjects and excluding subjects with obvious hyperinsulinaemia.**

Meal/Dose	Insulin AUC (0-2 hours) all subjects*		Insulin AUC (0-2 hours) excluding subjects with hyperinsulinaemia*		Insulin AUC (0-4 hours) all subjects		Insulin AUC (0-4 hours) excluding subjects with hyperinsulinaemia	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
control	14	3559 ± 1478	12	3179 ± 1107	14	4511 ± 1914	12	4016 ± 1459
LBG	14	3643 ± 1926	12	3079 ± 1332	14	4801 ± 2794	12	3990 ± 1962
MBG	14	3072 <sup>#</sup> ± 1432	12	2628 <sup>#</sup> ± 903	14	4351 ± 2395	12	3683 ± 1706
HBGO	14	2952 <sup>#</sup> ± 1664	12	2431 <sup>#</sup> ± 1079	14	4085 ± 2348	12	3353 ± 1543
HBGX	14	2959 <sup>#</sup> ± 1533	12	2471 <sup>#</sup> ± 944	14	4247 ± 2484	12	3514 ± 1652

\* RMANOVA  $p < 0.05$  for overall analysis

<sup>#</sup>  $p < 0.05$  for t-test comparison with control dose of fibre

**Table 4-4 Post-prandial glucose (mmol L<sup>-1</sup> X min), ghrelin (pg mL<sup>-1</sup> X min) and CCK (pg mL<sup>-1</sup> X min) responses**

Meal/ Dose	Glucose AUC (0-2 hours)		Ghrelin AUC (0-4 hours)		CCK AUC combined sexes (N=14)		CCK AUC female (N=7)*	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
control	14	505 ± 142	12	5320 ± 2850	14	3052 ± 1451	7	2213 ± 1227
LBG	14	493 ± 96	12	3860 ± 3217	14	3455 ± 1708	7	2670 ± 796
MBG	14	505 ± 83	12	5300 ± 4428	14	3680 ± 1652	7	3061 ± 960
HBGO	14	488 ± 109	12	5041 ± 3007	14	3831 ± 1872	7	3190 ± 1049
HBGX	14	493 ± 101	12	4408 ± 3724	14	3814 ± 1800	7	3478 ± 1289

\* RMANOVA  $p < 0.05$  for overall analysis

### 4.3.5 Subjective Satiety

Responses to individual VAS questions (Table 4.5) for varying  $\beta$ -glucan doses from the oat bran ingredient showed significant variation for Question 3 (How full do you feel?) ( $p=0.017$ , RMANOVA for AUC). Other questions approached a significance level of 0.05 ( $P=0.071$ ,  $P=0.101$ ,  $P=0.099$  for Questions 1, 2 and 4 respectively). Pairwise comparisons using Bonferroni adjustments indicated differences primarily between the control and all doses of fibre. Comparison between HBGO and HBGX responses showed some variations. Question 3 showed a difference ( $P=0.013$ ), where the HBGO product appeared to make participants feel more full. Data for all other questions showed a similar trend of increased satiety with HBGO, but no significant results were noted (Question 1,  $P=0.263$ ; Question 2,  $P=0.101$ ; Question 4,  $P=0.794$ ).

When the responses to questions were analysed as a single response (a measure of hunger with Questions 2 and 3 responses reversed), an overall effect was noted using RMANOVA ( $P=0.039$ ). Pairwise comparisons showed no difference between fibre doses (LBG, MBG and HBGO) but the overall effect resulted from differences between the control and all other fibre doses. No gender effect was noted. RMANOVA of the two HBG doses showed less hunger/greater fullness with the oat bran product (HBGO) but this only tended towards significance ( $P=0.085$ ). Student's t-test analyses of the control meal compared to other doses showed differences between the control and all oat bran doses with lesser significance with the HBGX ingredient ( $P=0.013$ , 0.026, 0.015, 0.086 for LBG, MBG, HBGO and HBGX respectively).

Cronbach's Alpha analysis for the oat bran doses indicated a high correlation between results for all four questions, with  $\alpha > 0.95$  for all fibre doses. This indicates that responses to each individual question were relatively indicative of the overall results when questions were combined.

**Table 4-5 Visual Analogue Scales Score<sup>i</sup>**

	<i>Control</i>	<i>LBG<sup>ii</sup></i>	<i>MBG<sup>ii</sup></i>	<i>HBGO<sup>ii</sup></i>	<i>HBGX<sup>ii</sup></i>
<b>Question 1</b> How hungry do you feel?	13600±3900	11100±4500	11420±4080	10660±5180	11700±3840
<b>Question 2</b> How satisfied do you feel?	9350±4630	11400±5060	10750±4400	11530±6080	10440±5110
<b>Question 3<sup>iii</sup></b> How full do you feel?	8600±4690	11470±5310	10480±4750	11460±6540	9630±5320
<b>Question 4</b> How much could you eat?	15000±4100	13230±4730	12840±4440	13350±5090	13530±4560
<b>Combined<sup>iii</sup> (Q2 &amp; Q3 reversed)</b>	58640±16580	49510±19150	51030±16480	49020±22310	53170±17920

<sup>i</sup> Area under the curve over 4 hours, AUC (mean ± standard deviation)

<sup>ii</sup> LBG = low β-glucan dose, MBG = mid β-glucan dose, HBGO = high β-glucan dose (all containing high β-glucan oat bran), HBGX = high β-glucan dose containing extracted β-glucan.

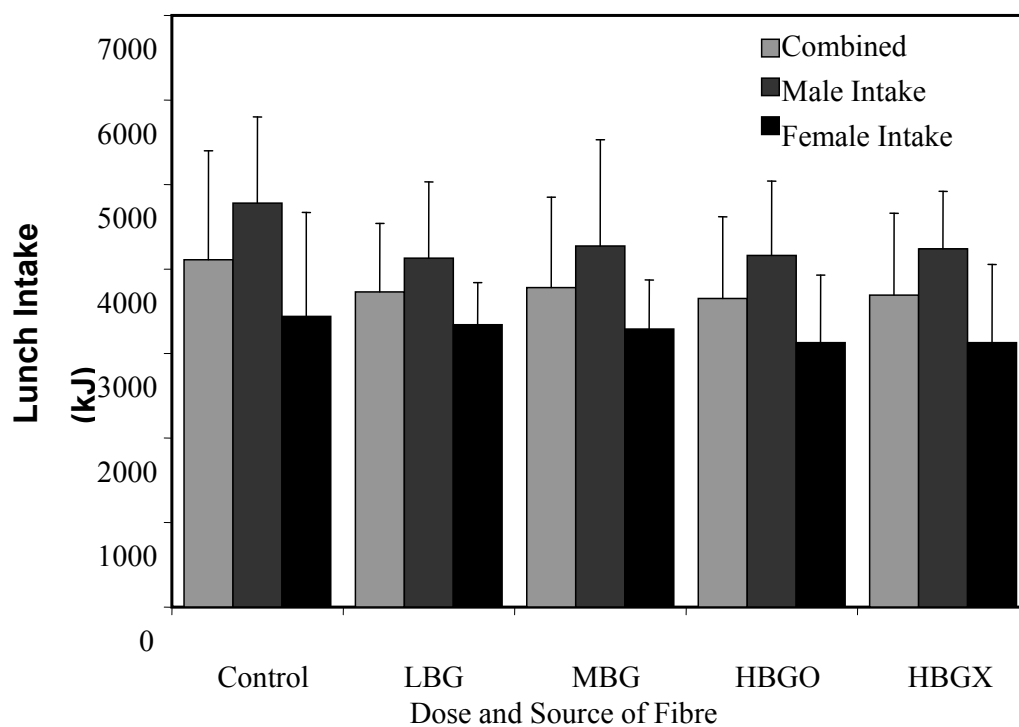
<sup>iii</sup> RMANOVA  $P < 0.05$

#### 4.3.6 Second Meal Intake

RMANOVA of lunch energy intake data showed non-significant results ( $P=0.22$ ) although the trend was as expected (Table 4.6 and Figure 4.4). Overall, the lunch time energy difference between the control and HBGO meal was 460kJ. Less than 40kJ difference was noted between the HBGO and the HBGX meals. Student t-test analyses showed significant difference between the control and HBGX groups' meals ( $P=0.033$ ) and approaching significance for comparison between the control and HBGO groups' meals ( $P=0.097$ ). No gender/fibre interaction could be identified. However, results for males showed a drop in intake between the control and all doses of β-glucan, while the female response was more attenuated, more evenly decreasing with increasing fibre dose (Figure 4.5).

**Table 4-6 Dietary intake after each of the five test meals**

<i>Fibre/Dose</i>	<i>Males &amp; Females Mean ± SD (kJ)</i>	<i>P value (t-test) control vs dose</i>	<i>Male Intake Mean ± SD (kJ)</i>	<i>Female Intake Mean ± SD (kJ)</i>
control	4110 ± 1290	-	4780 ± 1020	3440 ± 1230
LBG	3730 ± 810	.177	4130 ± 900	3340 ± 500
MBG	3780 ± 1070	.194	4270 ± 1260	3290 ± 580
HBGO	3650 ± 970	.097	4160 ± 880	3130 ± 800
HBGX	3690 ± 970	.033	4240 ± 680	3130 ± 925



**Figure 4.5 Average (N=14) energy intake (kJ) at lunch meal for males, females and combined sexes.**

LBG = low  $\beta$ -glucan dose, MBG = mid  $\beta$ -glucan dose, HBGO = high  $\beta$ -glucan dose (all containing high  $\beta$ -glucan oat bran), HBGX = high  $\beta$ -glucan dose containing extracted  $\beta$ -glucan.

#### 4.4 Discussion

This study combined a variety of measures for appetite and satiety within a single experimental design. Content analysis of the organoleptically acceptable products found that  $\beta$ -glucan was stable to the extrusion processes and maintained characteristics for attributable metabolic effects. MW remained high, with good water solubility at body temperature, ensuring maximum viscosity within the aqueous gut environment. Variability in the cholesterol-lowering action of  $\beta$ -glucan has been attributed to low molecular weight or viscosity after certain forms of processing<sup>110, 189</sup> so maintaining these properties is important.

The decrease in MW noted with increasing  $\beta$ -glucan is most likely secondary to the heat generated during extrusion. The more viscous the raw ingredients (higher concentrations of  $\beta$ -glucan) the slower the flow rate through the extruder and the greater the likelihood of heat degradation during processing. However, if these results are compared to others utilising a variety of processing techniques<sup>189</sup> extrusion still compares favourably. As the proportion of  $\beta$ -glucan in the cereal increased, the solubility of the  $\beta$ -glucan also increased, probably as a result of increased pressure in the extruder. Therefore, the increase in solubility, more than made up for the decrease in MW and the overall viscosity in the *in vitro* extract actually increased as the  $\beta$ -glucan dose increased. It will always be critical to measure parameters such as MW, viscosity and solubility in a new product but it would seem extrusion is acceptable downstream processing for  $\beta$ -glucan products.

Previous research using VAS suggests no need to standardise meals prior to meal-tests<sup>157</sup>. This was confirmed here, where no true correlations were found between 24-hr recall data prior to testing and fasting VAS scores, ghrelin or CCK. This does not mean that fasting measures of these scores or hormones are unrelated to longer term dietary behaviours, particularly as these behaviours impact on weight control.

A range of soluble fibres, including  $\beta$ -glucan have been shown to improve glycaemic responses<sup>88, 92</sup>, with each gram of  $\beta$ -glucan found to decrease glycaemic index by four units<sup>88</sup>. The food vehicle, however, may be important. Studies have found that high doses of  $\beta$ -glucan (>5g) in pasta<sup>190</sup> and in rye bread<sup>68</sup> did not improve glycaemic responses.  $\beta$ -Glucan depolymerisation has been shown to increase during processing of

pasta and bread<sup>189</sup>. However,  $\beta$ -glucan from barley, served as a hot cereal, produced a significant decrease in glucose with only 2g of  $\beta$ -glucan in women<sup>96</sup>. All of the test meals here produced blunted glucose responses when compared to a similar study<sup>111</sup>. It was expected that the 43g of available carbohydrate here would produce a greater elevation in blood glucose from baseline, particularly in the overweight/obese study sample. The small response for the control was unexpected but the overall low energy content of test meals may have made differentiation between meals difficult. It is also recognised that venous sampling of blood glucose results in more attenuated responses compared to capillary samples<sup>191</sup>.

Regardless of glucose response, the observation of decreased insulin release was a positive outcome, possibly through delayed rate of glucose delivery or specifically through actions of CCK<sup>115</sup>. The role of hyperinsulinaemia in the development of obesity or indeed hyperinsulinaemia as a consequence of obesity may be multifactorial. Karpe and Tan argue that hyperinsulinaemia may lead to lipolytic inhibition in adipose tissue<sup>192</sup> so any ingredient which decreases insulin secretion is helpful in minimising obesity-promoting effects. This is particularly promising given that other authors have demonstrated increased insulin responses with dairy products (included here with our cereal) yet increasing doses of  $\beta$ -glucan still produced lowered insulin responses<sup>193</sup>. Our results indicated a dose response to  $\beta$ -glucan with a dose greater than 3g demonstrating a consistent decrease in insulin secretion. This is consistent with other recently published data identifying a dose of 4g  $\beta$ -glucan was necessary to show insulin decreases<sup>194</sup>. Differences in insulin results for subjects with hyperinsulinaemia indicate that future studies should examine glucose, insulin and appetite responses in subjects with and without insulin resistance. Given the trend of insulin responses over four hours, it is likely that greater subject numbers would have also shown a significant difference over the longer time frame.

A decrease in ghrelin is associated with food intake, but here the  $\beta$ -glucan did not suppress ghrelin secretion in a dose responsive manner. Other studies<sup>144</sup> investigated the effect of insoluble wheat and insoluble oat fibres on secretion of ghrelin. The oat-fibre bread showed no decrease in ghrelin compared to controls. It would seem that both the insoluble fibre<sup>144</sup> and the soluble fibre used in this study do not alter ghrelin secretion at the levels provided.

The dose-response relationship between  $\beta$ -glucan and CCK in our study demonstrates a possible mechanism of satiety associated with increasing fibre intake. The link between fibre and CCK secretion has been previously established<sup>119, 120</sup>. The greater sensitivity in females compared to males is also consistent with the literature<sup>119, 195</sup> and use of fibre in foods targeted specifically at increasing satiety in women may be justified. The effective dose of close to 4g demonstrated here is consistent with advice from others<sup>71</sup> relating to gastrointestinal effects of oat bran. Given the t-test results for males were close to significant, and the overall RMANOVA for combined sexes shows increasing CCK with fibre close to significance ( $P=0.110$ ) it is likely that greater subject numbers would have allowed elucidation of the precise level of  $\beta$ -glucan required to show CCK differences in men, and a subject group of combined sexes.

Overall the VAS scores to rate hunger/appetite indicate that even relatively low doses of  $\beta$ -glucan (>2g) will give a decreased feeling of hunger. Of interest is the marginal differences identified between the oat bran ingredient and the extracted  $\beta$ -glucan HBG meals. While biochemical and subjective measures, measure different things, the majority of results here support higher  $\beta$ -glucan improving all markers and measures of satiety, and it is only the VAS results for the HBGX fibre here which are inconsistent.

The wide variety of factors affecting meal intake<sup>154</sup> may limit the ability of small studies to identify differences in dietary intake related to a single nutrient. Our study manipulated nutrient composition of meals to focus on the effect of fibre and in particular oat  $\beta$ -glucan as a variable in appetite control, but wide standard deviations (up to 29% in males) may have diluted results. Based on the differences in energy intake in this study a sample size of 37 would be required for this result to reach statistical significance in a paired design (80% power, alpha 0.05). Although overall the RMANOVA of results was not significant, post-hoc t-test analysis indicated differences with the HBGX ( $p<0.05$ ) and perhaps the HBGO ( $p<0.1$ ) meals. The fact that female intake produced flat responses demonstrates that large numbers of females would be required to demonstrate differences and, that other factors may attenuate intake. In particular, the widely held social belief that women are more likely to show restraint in a buffet situation (regardless of appetite) may be demonstrated here.

The kilojoule difference between control and HBG doses may not show statistical significance, but the absolute difference of greater than 400kJ at a single meal is of

clinical significance if these results could be repeated in a more powerful study. Future studies should record intake over the entire day. If no compensatory intake took place later in the day the difference would equate to a 100g weight loss each week if maintained daily.

Overall, the use of  $\beta$ -glucan in foods with a target market of individuals wishing to maintain or lose weight through appetite control is justified. Appetite suppressants such as CCK are released in response to  $\beta$ -glucan at a minimum dose of approximately 3.8g. Subjective ratings of hunger are improved at a minimum dose of 2.2g of  $\beta$ -glucan. Insulin responses relevant to the development of Type 2 diabetes are significantly decreased at a dose of at least 3.8g of  $\beta$ -glucan. Although minor differences may exist in the clinical effectiveness of  $\beta$ -glucan, either when extracted or from oat bran, both show favourable results. However, variation in results between foods tested here and in other studies, necessitates individual testing of all  $\beta$ -glucan products.



## PART 2 - PYY responses to varying doses of oat $\beta$ -glucan in humans

<sup>1</sup>The majority of this section is the substantive content of work submitted for publication: Beck, E.J., Tapsell, L.C., Batterham, M.J., Tosh, S.M. & Huang, X. F. (2009) Increase in PYY levels following oat  $\beta$ -glucan ingestion is dose dependant in overweight adults. Accepted to **Nutrition Research**, 22 September, 2009.

## Introduction

The meal test study described previously, recorded acute biochemical and subjective measures of satiety, followed by energy intake from a subsequent meal, after varying doses of  $\beta$ -glucan in extruded breakfast cereals.  $\beta$ -Glucan was found to decrease insulin secretion over 2 hours (RMANOVA,  $P=0.011$ ) in a dose responsive manner from 2.16 to 5.45 g  $\beta$ -glucan/serving ( $P=0.007$ ). CCK levels increased linearly over the same range of  $\beta$ -glucan concentrations ( $P=0.002$ ) in women. Subjective satiety was increased at a  $\beta$ -glucan dose of 2.2g ( $P=0.039$ ). Subsequent meal intake tended to decrease by greater than 400kJ with higher  $\beta$ -glucan dose ( $>5$ g). Animal studies in our laboratory also demonstrated weight loss, increased satiety, increased PYY<sub>3-36</sub> and down-regulation of mRNA expression for NPY and Y2 receptors with increasing  $\beta$ -glucan dose<sup>196</sup>.

PYY belongs to the pancreatic polypeptide family which includes pancreatic polypeptide and neuroptide-Y (NPY). All three peptides share a common tertiary structure. PYY is primarily secreted by endocrine cells in the distal small bowel and colon<sup>122</sup>. DPP-IV hydrolyses PYY and converts the precursor PYY<sub>1-36</sub> to PYY<sub>3-36</sub>. PYY<sub>3-36</sub> is an appetite suppressant which acts on NPY cells via the NPY Y2 (Y2) receptor in the medial part of the arcuate hypothalamic nucleus of the brain<sup>197</sup>. In humans, infusions of PYY<sub>3-36</sub> comparable to those after a meal result in decreased energy intake at subsequent meals compared to a control group<sup>198</sup>. In addition studies showing obese subjects are still sensitive to the effects of this hormone<sup>126</sup> make it not only an interesting target for research into mechanisms of satiety but obesity in general.

The aim of this study was to determine the dose response of PYY to increasing doses of  $\beta$ -glucan (approximately 2-6g) in the form of extruded cereals containing oat bran high in  $\beta$ -glucan. This work is an extension of the previously described meal-test study which was undertaken after results from the animal studies were available and because duplicate samples had been stored at -80 degrees celcius after the initial study.

## 4.5 Methods

The original study did not prepare the plasma samples for PYY<sub>3-36</sub> analysis as the correct inhibitors were not added. Therefore, control samples, together with three different doses of  $\beta$ -glucan in cereals manufactured using the oat bran ingredient described in Chapter 4, Part 1, were tested for total PYY in the current study. Full methodologies for the meal test study are described in the previous chapter.

PYY in these samples was tested using an enzyme linked immunosorbent assay (MPEZHPYYT66K-Millipore Human PYY (Total)) kit according to the standard protocols. Results for PYY values were entered into SPSS for windows, Version 15.0 for both raw data and trapezoidal area under the curve (netAUC- where values were corrected for baseline but areas below the baseline were also subtracted<sup>187</sup>).

Differences in raw data and netAUC results between the different doses of  $\beta$ -glucan were identified using repeated measures analysis of variance (RMANOVA) with post-hoc Bonferroni adjustments. Results for the control compared to each dose were also reviewed at individual time points using students' t-test.

## 4.6 Results

Results from only 13 of 14 subjects were included in this analysis as some values obtained for one subject were between ten and fifty-fold greater than other subjects. The data for this subject was excluded from the overall analysis on the basis of its implausibility.

Review of raw data (Table 4.7) corrected for baseline indicated a trend towards significance ( $P=0.131$ ) where an increasing dose of  $\beta$ -glucan resulted in a greater release of PYY (Figure 4.5). Post-hoc Bonferroni adjustments showed the majority of this trend was due to differences between the control and highest dose of  $\beta$ -glucan ( $P=0.072$ ). Large standard deviations indicate the large inter-individual variations. NetAUC results showed a similar overall trend ( $P=0.102$ ) while the post-hoc calculations showed a significant difference between the control and the HBGO dose ( $P=0.039$ ) (Figure 4.6). Regression analysis showed a significant correlation ( $P=0.003$ ) between plasma PYY and total  $\beta$ -glucan content ( $R^2=0.994$ ).

RMANOVA calculations of the difference in PYY values at the time-point immediately before the lunch (4 hours) showed a significant dose response ( $P=0.023$ ) with post-hoc identification of a significant difference between the control and HBG meal tests ( $P=0.036$ ). Students' t-tests between the control and each dose at this time, show a trend to difference between the control and MBG dose ( $P=0.074$ ) and a statistically significant difference between the control and HBG dose ( $P=0.006$ ). If data is analysed for the first 2 hours, although the HBG dose elicits the greatest peak change in PYY (31 pg/mL at 30 minutes), no significant difference between results is shown ( $P=0.435$ ). However, if the results for the second 2 hour period (2 to 4 hours post-meal) are reviewed, a significant difference is noted for the RMANOVA analysis ( $P=0.035$ ).

**Table 4-7 Mean PYY values (pg/mL, corrected for baseline) for  $\beta$ -glucan doses over time (N=13)**

	<i>Time (minutes)</i>	<i>Minimum</i>	<i>Maximum</i>	<i>Mean</i>	<i>Std. Deviation</i>
Control	0	0	0	0	0
	15	-32.6	67.9	12.8	32.0
	30	-29.6	44.8	4.5	23.4
	60	-102.3	57.5	-0.4	42.1
	120	-94.0	59.1	-4.0	45.7
	180	-91.3	44.3	-20.0	40.8
	240	-110.6	38.1	-24.6	37.2
LBG	0	0	0	0	0
	15	-96.0	95.1	4.8	49.6
	30	-85.3	63.2	4.6	44.5
	60	-67.9	111.7	10.0	49.1
	120	-80.5	73.0	13.2	42.1
	180	-68.9	48.5	-6.6	36.2
	240	-104.3	57.0	-8.2	48.2
MBG	0	0	0	0	0
	15	-19.4	145.2	20.2	42.7
	30	-51.2	52.5	13.2	27.0
	60	-30.6	46.8	7.4	25.1
	120	-44.3	37.9	8.8	24.8
	180	-106.9	98.0	11.4	55.9
	240	-112.6	55.3	-0.1	47.0
HBG	0	0	0	0	0
	15	-48.3	50.9	17.4	24.6
	30	-100.9	247.5	31.1	76.7
	60	-58.4	198.0	22.8	66.2
	120	-104.1	185.2	25.0	64.2
	180	-58.4	64.0	13.1	33.1
	240	-19.8	84.2	17.5	28.6

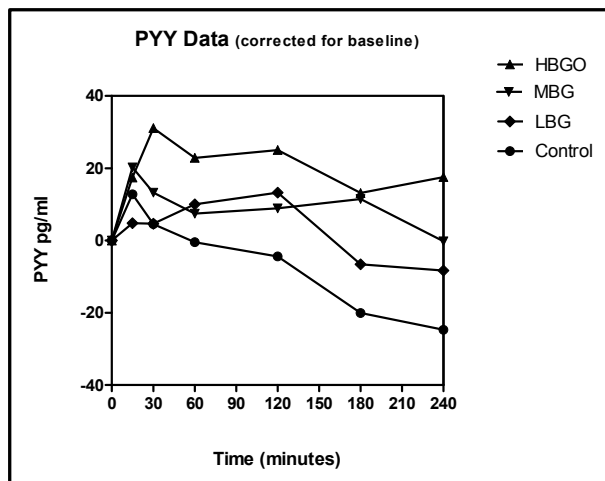


Figure 4.6 PYY responses corrected for baseline.

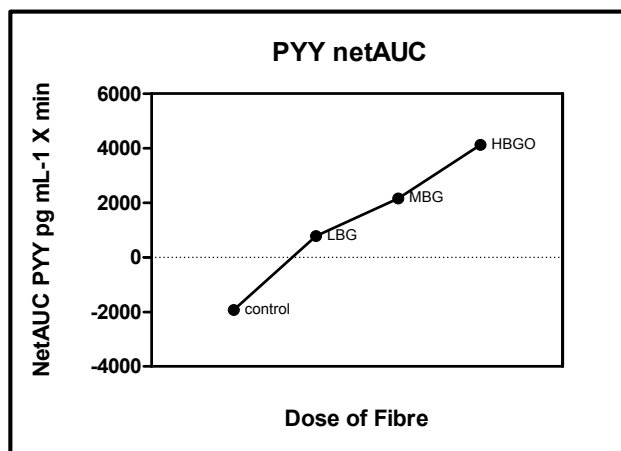


Figure 4.6 PYY netAUC for each test meal.

## 4.7 Discussion

This study found total levels of plasma PYY increase in a linear fashion with increasing concentration of  $\beta$ -glucan (up to 5.45g of  $\beta$ -glucan) in the first 4 hours after a meal. The strong correlation between meal-test PYY response and concentration of  $\beta$ -glucan indicate that it is affected acutely by the amount of soluble fibre, or at least the concentration of the tested oat bran. Post-hoc analysis indicated a difference between the control and the HBG dose of  $\beta$ -glucan and this is consistent with the literature which discusses a minimum level of between 4 and 6g as necessary for the gastrointestinal effects of  $\beta$ -glucan <sup>71</sup>.

Examining the data at different time-points shows a significant difference in PYY secretion over the longer time frames (two to four hours). In particular the single data point at four hours shows the greatest PYY level for the HBG dose. This is consistent with the secretion of PYY in the distal gut and colon and emphasises the longer lasting effects of anorexigenic hormones such as PYY. Studies over shorter time frames may be suitable for measuring glycemic and insulinemic advantages of  $\beta$ -glucan ingestion, however longer time frames may be required to show the full satiety effect of highly viscous fibres such as  $\beta$ -glucan.

The time frame for PYY increases also explains how studies of low viscosity versus high viscosity  $\beta$ -glucan <sup>105</sup> may show lower hormone responses over 2-3 hours, where the faster transit of the low-viscosity fibre causes higher levels of hormones such as PYY to be released initially. However, if results are reviewed for the entire day, a lower kilojoule intake is shown with high viscosity fibre ingestion <sup>105</sup>. This is also consistent with the trends shown in our original work for dietary intake <sup>199</sup> where the greater viscosity seen with the higher concentrations of  $\beta$ -glucan gave the lowest second meal energy intake. It is likely that the undigested nutrients in the large bowel caused by viscous soluble fibres result in long lasting satiety through the action of hormones such as PYY. They do not necessarily show increased satiety initially, and future studies should include dietary intake measured over an entire day. In addition, the fact that insulin secretion is decreased by  $\beta$ -glucan ingestion over 2 hours <sup>88, 199</sup> may result in a transient decrease in satiety <sup>150</sup>. This means that the benefits of  $\beta$ -glucan are wide ranging, where satiety hormone responses compensate for glycaemic mechanisms of control over intake.

The primary limitation of this study is the power, which was originally calculated to detect hormone changes such as CCK, as well as subjective satiety (Chapter 4, Part 1<sup>199</sup>), where 8-15 subjects should have identified differences between doses<sup>157</sup>. The large standard deviations in responses indicate that inter-individual variation is large and greater numbers are required to identify statistically significant results at all levels of analysis.

Previous unpublished animal data<sup>200</sup> showed increased circulating PYY<sub>3-36</sub> with increasing  $\beta$ -glucan dose ingested chronically. It would seem that acute exposure to  $\beta$ -glucan changes the levels of PYY released in humans consistent with the animal studies. Combining this new knowledge with previous studies it can be concluded that the optimal dose of  $\beta$ -glucan affecting satiety and other markers of appetite regulation would be between 4-6 g. The effects on satiety related hormones appear to be mediated through both viscosity and concentration. Acute studies relating to appetite should determine hormone levels for a minimum of four hours.



## CHAPTER 5      LONG TERM EFFECTS OF OAT $\beta$ -GLUCAN IN AN ENERGY-RESTRICTED DIETARY INTERVENTION

The majority of this Chapter is the substantive content of the work, Beck, E.J., Tapsell, L.C., Batterham, M.J., Tosh, S.M. and Huang, X-F. “Oat  $\beta$ -glucan supplementation does not enhance the effectiveness of an energy-restricted diet in overweight women”, accepted to **British Journal of Nutrition** 10 October 2009.

Discussion relating to the development of fibre products to meet dietary targets was included in, Beck, E., Tapsell, L., Batterham, M. and Huang, X-F. Developing Foods with Fibre Effects. 2008 **International Congress of Dietetics**, Yokohama, Japan. (OS-8-1, Sept 8-11).

Discussion relating to dietary intervention effects on consumption of bread and cereal products was included in, Beck, E.J., Tapsell, L.C., Tosh, S.M., Batterham, M.J. and Huang, X. Dietary interventions with fibre ingredients – considerations, measurements and manipulations. 2009 **Cereal Foods World**, 54(4):A16. *Abstract for oral presentation.*

## 5.1 Introduction

As the obesity epidemic escalates food industry hurries to provide options for consumers who at least espouse a desire for “healthy foods”, including those which will help with weight control or weight loss. Researchers turn first to dietary patterns which provide epidemiological evidence for health benefits, such as linking a Mediterranean diet with lowered cardiovascular disease. Secondly, researchers dissect the designated diet down to foods which make up such a meal pattern, such as reviewing benefits specifically of olive oil, or consumption of salad foods. Finally the food is broken down to more basic constituents such as monounsaturated fats, omega-3 polyunsaturates or anti-oxidants. Including these ingredients in a meal plan may or may not provide the same benefits as the original diet. However importing a beneficial item into a different cultural food system may provide at least some health advantages to consumers and also marketing advantages to food industry.

Large bodies of epidemiological data show an inverse relationship between dietary fibre intake and body weight<sup>10, 39, 42, 45, 46</sup> so the logic and simplicity of including fibre in an energy-controlled diet remains tantalising. As a part of food, fibres are found in a fermentable (soluble) or non-fermentable (insoluble) form, but early research has been unable to show benefits from including either form in short term (3-4 weeks) *ad libitum* diets<sup>95</sup>. This was also the case when the form of fibre was  $\beta$ -glucan, a soluble fibre delivered by oats<sup>100</sup>, well recognised for its cholesterol<sup>80</sup> and glucose lowering<sup>88</sup> actions. In order to better expose the advantages apparent from epidemiological studies, more work is required in understanding the physical features of fibre, the food delivery system, and how these may work together to affect mechanisms associated with weight management, such as satiety, particularly over longer periods of time.

It is accepted that soluble fibre, by its viscous nature will not only increase upper gastrointestinal transit time, but also stimulate cholecystokinin (CCK) which will increase peristalsis<sup>71</sup>. The effects of other hormones are less clear, but the ileal-brake

formed by undigested foods in the distal gut (occurring with high fibre foods), in addition to the fermentation of soluble fibres in the large bowel are all seen as positive benefits of fibre overall. Meal studies are able to expose these mechanisms of action. This is done via assessment of biochemical markers and subjective measures of satiety, in addition to monitoring subsequent food intake after consumption of the test food. Studies of  $\beta$ -glucan have identified doses as low as 2 g may elicit acute lowering of glycaemia<sup>96</sup> while others suggest a minimum of 4 g<sup>71</sup> may be required for other gastrointestinal effects, such as those causing the release of appetite hormones. However, meal test studies only define an acute situation in very controlled conditions.

In addition to the time factor, the food delivery system requires consideration. For example, the clinical effectiveness of fibres such as  $\beta$ -glucan may be reduced in certain foods. It has been shown that in bread making, endogenous enzymes in the bread reduce the viscosity of the  $\beta$ -glucan thereby decreasing its clinical effectiveness<sup>201</sup>. However, a recent satiety study<sup>105</sup> looking at high dose (10g)  $\beta$ -glucan and appetite hormones showed that a lower viscosity drink (with viscosity lowered using  $\beta$ -glucanases) increased levels of certain appetite hormones such as CCK and GLP-1 compared to the high viscosity version of the same drink. Nevertheless, such contrasts once again do not define what may happen when soluble fibres are consumed over time, as a change in hormones over a few hours does not necessarily translate to appetite and weight changes over a longer period of time.

The current study describes a 3-month randomised controlled dietary intervention trial, designed to review the specific effects  $\beta$ -glucan incorporated in an energy-restricted meal plan. The primary outcome was a difference in weight reduction between the control group and intervention groups. Secondary outcomes included a variety of hormones linked with satiety or change in body weight, subjective satiety measures and perceived satisfaction with the product. We hypothesised that the subjects receiving  $\beta$ -glucan would lose more weight than the subjects on the control diet and that changes in appetite hormones may be detected due to mechanisms linking satiety with ingestion of  $\beta$ -glucan.

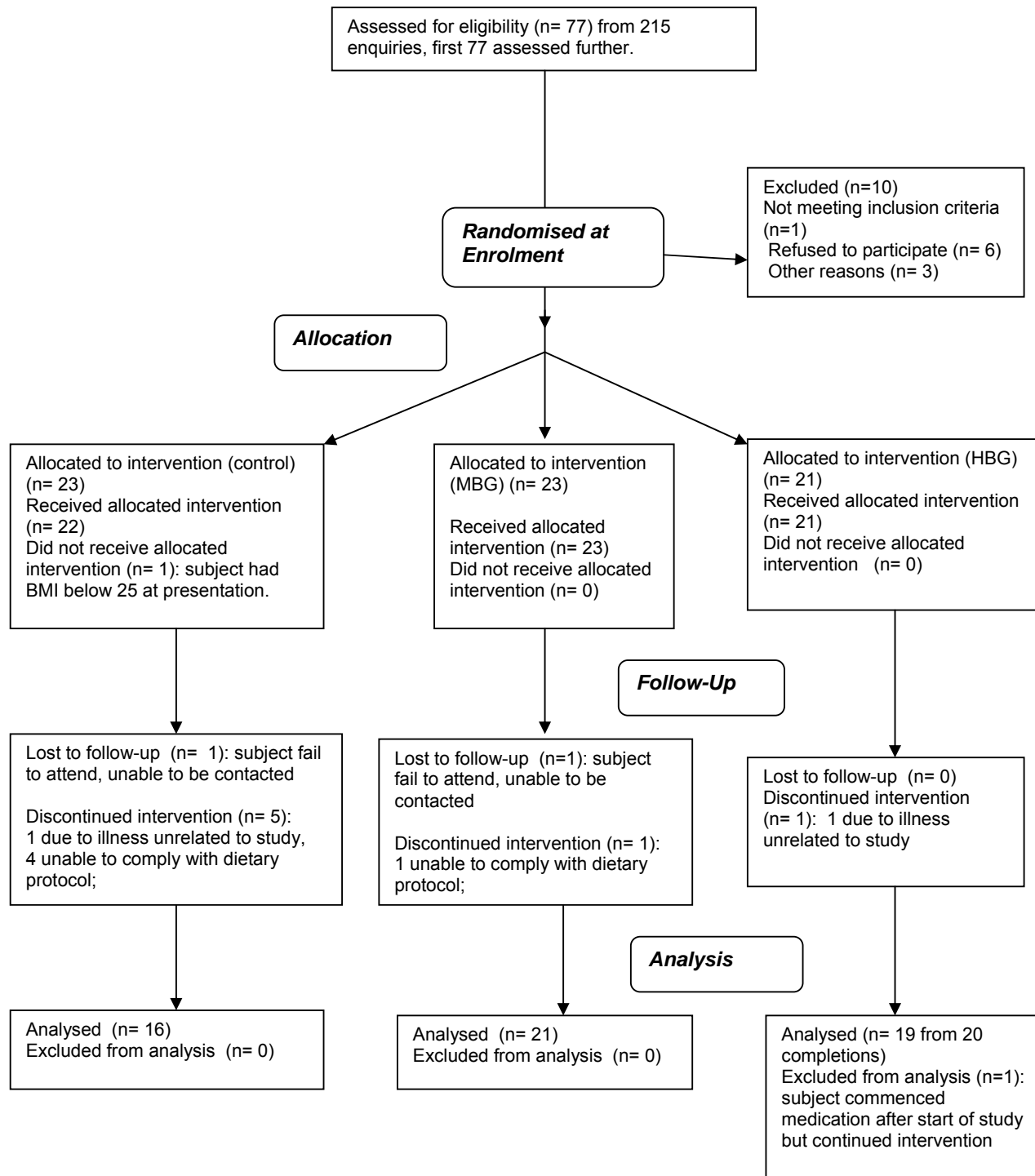
## 5.2 Methods

### 5.2.1 Subjects and Recruitment

This was a 3-month parallel randomized controlled trial with female subjects, based on evidence that they may exhibit greater acute hormone changes with fibre intake<sup>119</sup>. There were three arms to the study with all groups receiving advice on energy restriction and all subjects receiving cereal products to include in their diets. The control group had relatively high fibre products with no  $\beta$ -glucan while the intervention groups had similar products with added  $\beta$ -glucan at a moderate (5-6g/day - MBG) and high (8-9 g/day - HBG) level. A sample size of 20 subjects per group was based on data from a previous study showing a 1.8 kg difference between a group supplemented with  $\beta$ -glucan and a control group where both groups were consuming a calorie deficient diet<sup>202</sup>. For a power of 80%, 17 subjects would be required in each group for this change to be significantly different at an alpha level of 0.05, and recruiting at least 20 subjects would allow for dropouts. Inclusion criteria advertised in local media were 19-45 years of age (pre-menopausal), body mass index range from 25-32 kg/m<sup>2</sup>, non-smokers, no known food allergies and of general good health. Ethical clearance was given by the University of Wollongong, Human Ethics Committee (HE06/311) and the trial registered with the Australian Clinical Trials Registry (ACTRN12607000126415).

The first 77 of 215 enquiries were screened resulting in randomisation of 66 subjects, using a computer-generated sequence using random permuted blocks (Figure 5.1). All subjects, but not dietitians were blinded to their randomisation status within the trial. Subjects attended the study centre a total of five times. Visits included collection of background dietary data using a validated diet history interview<sup>203</sup> and instruction on completion of a 3-day weighed food record, collection of fasting blood samples at baseline together with dietary education, two follow-up dietary visits and collection of fasting blood samples at 3 months.

Figure 5.1 Flow diagram of participation in the study



All subjects had their height, weight and waist circumference recorded at each visit to the centre. Weight was recorded using Tanita Scales (Model No. UM-019). Percentage body fat was measured using Impedimed bioimpedance device (Imp SFB7 for Body Composition). Subjects were also contacted by telephone on one occasion in-between the monthly visits to provide dietary review and support. Visual analogue scales (VAS) related to appetite<sup>157</sup>, food records and the Baecke Physical Activity Questionnaire<sup>204</sup> were all completed at baseline, half-way through the study and within the final week of the study. For the VAS, subjects recorded their feelings on individual forms at six different time-points throughout the day – immediately before each of the three main meals and 2 hours after each of these meals. Markings by participants were measured in millimeters. All nutritional analysis was performed using FoodWorks 2007, Version 5, Xyris Software, Brisbane, Australia, with nutrient contents of study foods added as required.

### 5.2.2 Dietary Intervention

A basal metabolic requirement for energy was calculated for each subject using the Schofield equation using body mass index (BMI) equal to 25 kgm<sup>-2</sup>. A low activity factor (1.3) was chosen to estimate energy requirements and then 2000kJ was subtracted from this level to a calculated weight loss of 0.5 kg/week. Intervention diets were designed to control for all macronutrients with the only variation in total fibre, primarily  $\beta$ -glucan such that a typical diet, as described in Table 5.1 only varied in the trial products given. Product development for cold and hot cereals as well as additional snack items took place in a pilot processing plant by qualified cereal food technologists.  $\beta$ -glucan was sourced from a commercial refined oat bran ingredient. Subjects were instructed to eat the cereal provided at breakfast (RTE cereal or porridge) and two snack items at afternoon tea (choice of 2 muesli bars, 2 cereal snack-packs or one muesli bar and one cereal snack-pack). Product consumption was evaluated for overall satisfaction and observed positive and negative symptoms using questionnaires at the completion of the study.

Products were analysed for protein, fat, carbohydrate, total fibre, sodium and energy by Bread Research Institute Pty Ltd, North Ryde, 2113, Australia. The results were adjusted for portion size and the following data obtained (Table 5.2). A full ingredients list is included in Appendix 5-1. Products were tested for molecular weight, solubility,

viscosity and both soluble and total  $\beta$ -glucan content by Dr Susan M. Tosh at Agriculture and Agri-Food Canada. A general methodology is described below.

**Table 5-1 Food serves for typical dietary study participant**

<i>Food group</i>	<i>Serve Size</i>	<i>Number of serves/day</i>
Breads/cereal/ starchy vegetables	1 slice bread or ½ cup pasta or 1 medium potato	4 serves
Other vegetables	½ cup cooked = 1 cup raw	2.5 cups cooked or 5 cups raw
Fruit	1 piece fresh or ¾ cup canned	2 pieces
Milk/ alternatives	150mL light/200mL skim, 100g lowfat yoghurt	2-3 serves
Meat/ alternatives	30g meat/45-60g fish/ 20g low fat cheese	3-4 serves
Fats	1 tsp oil or margarine or 1 tbsp avocado	3-4
Control or intervention products	Portion controlled pre-packed	Cereal + 2 snacks (muesli bar or snackpack of cereal)

The total  $\beta$ -glucan content was determined following the method of Glennie-Holmes and McCleary<sup>182</sup> using a kit from Megazyme Inc. (Megazyme International, Bray, Ireland). An *in vitro* digestion protocol of Beer<sup>183</sup> was used to determine soluble  $\beta$ -glucan. The peak molecular weight of the soluble  $\beta$ -glucan was measured by high performance liquid chromatography (HPLC) with a size exclusion column using calcofluor detection<sup>185</sup>. The apparent viscosity of the extract was determined with a controlled strain rheometer (A-RES, TA Instruments, NJ) with comparisons between samples using the apparent viscosity at 30 s<sup>-1</sup>.

**Table 5-2 Nutrient composition of trial products**

<i>Name</i>	<i>Control Cereal</i>	<i>Control Porridge</i>	<i>Control Snack</i>	<i>Control Bar</i>	<i>MBG Cereal</i>	<i>MBG Porridge</i>	<i>HBG Cereal</i>	<i>HBG Porridge</i>	<i>HBG Snack</i>	<i>HBG Bar</i>
<i>Serving g</i>	48	49	20	24	49	50	49	55	23	23
<i>kJ</i>	715	730	337	355	755	740	701	787	336	336
<i>Protein g</i>	3.4	3.4	2.1	1.8	5.3	4.9	5.9	6.3	2.6	2.5
<i>Fat g</i>	0.8	0.8	1.2	0.7	2.8	2.5	1.6	2.7	0.6	2.3
<i>Sat. g</i>	0.2	0.2	0.6	0.3	0.5	.5	0.3	0.6	0.2	0.9
<i>Poly. g</i>	0.4	0.2	0.3	0.2	1.1	1.0	0.6	1.0	0.2	0.3
<i>Mono. g</i>	0.2	0.3	0.3	0.2	1.2	1.1	0.6	1.2	0.2	1.1
<i>CHO g</i>	35.0	37.3	15.2	16	29.4	30	26.9	29.7	14	10.8
<i>Sugars g</i>	13.7	19.8	6.3	5.4	11.5	16.8	13.4	14.1	4.5	3.5
<i>Fibre g*</i>	4.0	1.2	0.6	2.9	7.4	6.6	10.1	9.7	4.4	4.6
<i>Na mg</i>	79	18	39	19	154	3	152	2	43	41
<i>Moisture%</i>	4.1	5.4		2.1	2.7	5	3.2	5.6	1.1	2.8

**\*total fibre**



The amount of  $\beta$ -glucan in the control products was negligible ( $<0.2\text{g/serve}$ ).  $\beta$ -glucan contents of the products, including soluble  $\beta$ -glucan are included in Table 5.3. The  $\beta$ -glucan in the ready-to-eat (RTE) cereal and snack products had a higher solubility than the other products, 58.3 to 70.1% with solubility improved by extrusion (porridges not extruded). The solubility of the  $\beta$ -glucan in the porridge samples was approximately 38% for the MBG and HBG samples. The solubility of the  $\beta$ -glucan in the cereal bars was between that of the extruded product and the porridge. The molecular weight of  $\beta$ -glucan extracted from all of the products showed only limited decreases due to effects of processing. It was highest where there was least processing (muesli bars and porridge MW approximately 3 000 000 g/mol) and lower in the extruded products (RTE cereal and cereal snack MW approximately 1 500 000 to 2 000 000 g/mol).

The cereal and porridge samples with lower  $\beta$ -glucan contents (MBG samples) produced lower viscosity extracts (Table 5.3). The viscosity profiles of the HBG muesli bar and the HBG porridge had extracts with very similar viscosity profiles despite their differences in total  $\beta$ -glucan content. This is due to the greater solubility of the  $\beta$ -glucan in the muesli bar. The HBG RTE cereal had the greatest viscosity due to its high  $\beta$ -glucan and high solubility.

**Table 5-3  $\beta$ -glucan and total fibre content of study products (allowing for moisture and fruit content, calculated for serve size).**

<i>Sample</i>	<i>Description</i>	<i>Soluble <math>\beta</math>-glucan g per serve</i>	<i>Total <math>\beta</math>-glucan g per serve</i>	<i>Viscosity of Extract (mPa.s)</i>	<i>Total Fibre content (g)</i>
Control	RTE Cereal	Negligible	Negligible	Not done	4.0
Control	Porridge	Negligible	Negligible	Not done	1.2
Control	Extruded Snack	Negligible	Negligible	Not done	0.6
Control	Muesli Bar	Negligible	Negligible	Not done	2.9
MBG	RTE Cereal	2.3	3.9	14.6	7.4
MBG	Porridge	1.2	3.0	19.0	6.6
HBG	RTE Cereal	3.5	4.8	83.9	10.1
HBG	Porridge	1.7	4.4	58.9	9.7
HBG	Extruded Snack	1.4	2.1	54.5	4.4
HBG	Muesli Bars	0.9	1.7	57.0	4.6

Abbreviations: RTE, ready to eat; MBG, mid-dose  $\beta$ -glucan; HBG, high dose  $\beta$ -glucan

All of the test products showed physico-chemical characteristics which would be expected to produce bioactivity. The molecular weights remained greater than one million and the solubilities were greater than 35%. These products should have increased viscosity in the upper digestive tract which should modify the digestion and absorption rates of the nutrients.

### 5.2.3 Clinical Indices

Fasting blood samples at 0 and 3 months were collected using Sarstedt™ Monovette Blood Collection tubes. Glucose, insulin, total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and triglyceride (TG) analysis was performed at an accredited pathology laboratory (Southern IML Pathology, Wollongong, Australia). A further sample was collected into tubes containing potassium EDTA (to achieve a concentration of ½-2 mg EDTA/ml of blood after collection). Both tubes contained dipeptidyl peptidase IV (DPP-IV – Millipore) to level of 10 uL per mL of blood. One tube also contained aprotinin equivalent to 0.6 TIU per mL of blood (Aprotinin from bovine lung Sigma Aldrich A1153 dissolved in normal saline with 0.9% benzyl alcohol) for analysis of CCK and PYY<sub>3-36</sub>. Together with the DPPIV inhibitor the second tube contained 240uL of complete protease inhibitor cocktail (Roche, Australia) made to 25X concentration in distilled water. Final concentrations met the recommendations for the manufacturers' protocols for assays described below. The blood samples were then centrifuged at 4 degrees Celsius for 15 minutes at 1500xg. The plasma was stored at -80 degrees celcius until further analysis could be completed.

Ghrelin, leptin, GLP-1 and total PYY were analysed using a Lincoplex™ Human Gut Hormone Panel (Cat # HGT-68K) according to manufacturers instructions. CCK analysis used Phoenix Peptides™ radioimmunoassay (RK-069-04) for cholecystokinin octapeptide (CCK 26-33). The standard radioimmunoassay protocol was applied but without extraction of the peptides prior to the assay. PYY<sub>3-36</sub> analysis utilized a human specific radioimmunoassay sourced from Linco (PYY-67HK) using the standard protocol.

### 5.2.4 Statistical Analysis

Data for all anthropometry, blood analysis, VAS measurements and dietary intake were entered into SPSS for windows, Version 15.0 (SPSS 15.0, Chicago, Illinois). Repeated measures analysis of variance (RMANOVA) using the general linear model with group (control, MBG, HBG) 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. Log (base 10) transformations were also used as appropriate. Regression analysis was used to identify correlations between groups between anthropometric and biochemical indices. One-way ANOVA was used to review VAS and Baecke questionnaire results at various time-points identifying any differences between the groups.

## 5.3 Results

### 5.3.1 Baseline Data

Final numbers in each group were 16 controls, 21 MBG and 19 HBG (Figure 5.1). There were two withdrawals each from the MBG and HBG groups but six from the control group. Fifty-six subjects were included in the final data analysis. Some subjects did not complete all forms or blood was unable to be drawn for certain tests, so numbers in each calculation varied.

At baseline, there were no significant differences between group anthropometric and metabolic measures (Table 5.4). Subjects were overweight but overall were not hyperlipidaemic (mean cholesterol  $5.03 \pm 1.07$  mmol/L). However, 47 of 55 subjects whose cholesterol was recorded had a cholesterol greater than the 4.0 mmol/L Australian National Heart Foundation guidelines for prevention of coronary heart disease. Of these, 16 had cholesterol levels greater than the traditional guideline of 5.5 mmol/L. The only baseline biochemistry measure which showed significant differences between groups was the fasting blood glucose level (BGL) ( $p=0.046$ ) where the level for the HBG group was lower than that of the other two treatments (Table 5.4).

**Table 5-4 Baseline characteristics of study subjects.**

<i>Variable</i>	<i>Control (n=16)</i>	<i>MBG (n=21)</i>	<i>HBG (n=19)</i>	<i>P-value</i>
Age (years)	37.1 ± 5.6	37.7 ± 6.0	37.4 ± 5.5	0.96
Weight (kg)	77.0 ± 7.8	80.9 ± 8.2	77.6 ± 6.5	0.17
BMI (kg/m <sup>2</sup> )	29.2 ± 2.2	29.3 ± 2.2	29.3 ± 2.1	0.86
Waist (cm)	83.8 ± 5.7	85.1 ± 8.0	82.4 ± 5.6	0.29
Fasting blood glucose (mmol/l)	4.7 ± 0.6	4.9 ± 0.5	4.5 ± 0.4	0.05*
Fasting insulin (μU/l)	10.7 ± 4.3	11.1 ± 5.8	12.1 ± 4.8	0.57
Cholesterol (mmol/l)	5.2 ± 0.7	5.0 ± 1.1	4.9 ± 1.3	0.73
LDL (mmol/l)	3.2 ± 0.5	2.9 ± 1.0	2.7 ± 0.9	0.17
HDL (mmol/l)	1.6 ± 0.4	1.6 ± 0.3	1.7 ± 0.3	0.63
TG (mmol/l)	1.3 ± 0.4	1.1 ± 0.5	1.0 ± 1.0	0.55

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein, TG, triglyceride.

\* Significant (analysis of variance)

Review of food records and diet histories at baseline indicated relatively well matched groups. No significant differences were noted between energy, fibre, protein, fat or carbohydrate overall (Table 5.5). The only significant difference at baseline was the amount of monounsaturated fats consumed ( $p=0.025$ ) where post-hoc analysis using Bonferroni adjustments showed significantly less monounsaturated fat (MUFA) in the MBG group ( $24.8 \pm 9.8\text{g}$ ) compared to the control ( $32.5 \pm 8.0\text{g}$ ). The MUFA intake of the HBG group ( $28.8 \pm 6.8\text{g}$ ) was between these two levels.

### 5.3.2 Dietary Intervention

Evaluation of the food records at mid-way through the study and at the end indicated little variation between the groups for macronutrient intakes at each time point with no significant differences between groups. Review of the overall dietary composition of baseline diets compared to the mid-point of the study and the 3-month end-point showed a decrease in percentage energy from fat, and increase in the percentage energy from protein and carbohydrate (Table 5.5). General compliance with a weight reduction regimen was identified by overall weight loss (described below) and energy restriction detailed in the food records. Total energy intake was significantly lower at the mid-point (mean  $6308 \pm 1068$  kJ) of the trial and the end-point (mean  $6000 \pm 1163$  kJ) ( $p<0.001$ ) compared to baseline (mean  $8725 \pm 1703$  kJ) using RMANOVA with fibre level as the between group effect and time as the within-subject variant. There was no interaction effect over time ( $p=0.192$ ) indicating all groups followed the energy restricted diet to the same extent.

Dietary compliance with the study products measured by review of 3-day food records indicated use of the breakfast cereal was very high (90% consumed) while compliance with the snacks was reasonable (74% consumed). Compliance with the breakfast cereal was very high while compliance with the snacks was reasonable (Table 5.6).

Calculation of fibre values between the three groups indicated that the differences between the groups could be accounted for by the difference in fibre between the groups product supply. This includes both soluble ( $\beta$ -glucan) and insoluble fibre from the products. Overall, the food records indicated that the groups achieved a significant difference in the amounts of dietary fibre consumed at mid-point ( $p < 0.001$ , RMANOVA) and the last week of the study ( $p < 0.001$ , RMANOVA). There was limited change in the control group, indicating that the products supplied to subjects (which especially in the case of the cereal were relatively high fibre) replaced foods of similar fibre content.

**Table 5-5 Reported energy and macronutrient intakes at baseline, mid-point and 3 months, with P values for RMANOVA between groups.**

<i>Variable</i>	<i>Baseline</i>			<i>Mid-point</i>			<i>3 months</i>			<i>P-values</i>		
	<i>Control</i>	<i>MBG</i>	<i>HBG</i>	<i>Control</i>	<i>MBG</i>	<i>HBG</i>	<i>Control</i>	<i>MBG</i>	<i>HBG</i>	<i>Group</i>	<i>Time</i>	<i>Interaction</i>
Energy (kJ)	9229±1419	8394±2122	8213±1284	6060±910	6480±1080	6008±1060	6090 ± 1089	6193 ± 1226	5496 ± 1108	0.106	<0.001*	0.192
Protein (%E)	18.6 ± 3.5	18.5 ± 5.1	17.6 ± 5.6	20.6 ± 2.7	21.3 ± 3.7	21.8 ± 6.1	19.3 ± 3.6	20.6 ± 3.7	21.3 ± 3.7	0.411	<0.001*	0.363
CHO (%E)	42.9 ± 6.5	42.0 ± 16.4	42.1 ± 8.8	50.3 ± 7.7	44.7 ± 8.8	47.5 ± 8.1	50.3 ± 11.2	45.2 ± 4.3	49.3 ± 10.4	0.217	<0.001*	0.842
Total Fat (%E)	34.9 ± 19.0	32.1 ± 10.1	34.3 ± 9.1	22.6 ± 8.8	27.0 ± 8.9	23.5 ± 7.2	22.4 ± 7.9	26.9 ± 6.5	22.7 ± 8.4	0.321	<0.001*	0.062
SFA (%E)	13.3 ± 3.8	12.7 ± 5.0	14.5 ± 3.8	8.4 ± 3.2	9.5 ± 4.0	8.7 ± 3.3	8.6 ± 3.8	9.7 ± 2.5	7.9 ± 3.2	0.547	<0.001*	0.217
PUFA (%E)	4.6 ± 1.4	4.4 ± 1.7	5.2 ± 1.8	3.7 ± 1.6	4.2 ± 0.6	3.5 ± 1.2	3.6 ± 1.6	4.0 ± 1.3	3.8 ± 1.3	0.458	<0.001*	0.202
MUFA (%E)	13.1 ± 3.2	10.9 ± 4.1	13.0 ± 3.1	8.1 ± 4.4	10.0 ± 3.7	8.9 ± 3.0	7.7 ± 3.0	9.8 ± 3.0	9.0 ± 3.8	0.645	<0.001*	0.006*
Total Fibre (g)	21.9 ± 4.6	21.2 ± 6.0	20.5 ± 6.8	24.2 ± 5.4	28.0 ± 4.2	34.1 ± 6.9	21.6 ± 4.5	27.4 ± 5.6	33.0 ± 3.9	<0.001*	<0.001*	<0.001*

Abbreviations: E, energy; CHO, carbohydrate; SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids.

Data are means ± s.d.

\* Significant (repeated measures analysis of variance)

**Table 5-6 Intake of study products calculated from food records.**

	<i>Group</i>	<i>Snacks (average serves/day) (Total of 2 equals 100%)</i>	<i>Cereal (average serves/day) (Total of 1 equals 100%)</i>
Mid-point Results	Control	1.48	0.95
	MBG	Control Snack 0.76	
		HBG Snack 0.89	0.92
	HBG	1.33	0.93
Final Results	Control	1.37	0.94
	MBG	Control Snack 0.71	
		HBG Snack 0.81	0.89
	HBG	1.58	0.94

Table 5.7 demonstrates that the differences in fibre consumption between the control and intervention groups, is due specifically to the differences in fibre in the study products the subjects were supplied with. It also shows that the fibre difference for the MBG group was almost completely accounted for by the variation in  $\beta$ -glucan content and that 67-70% of the difference in the HBG group was due to  $\beta$ -glucan. It is most likely the remaining fibre difference is due to the insoluble fibre which was provided in the products, contained in the raw  $\beta$ -glucan ingredient. Total fibre differences between the control and MBG groups were completely accounted for by the differences in fibre in the products. However, the different contributions from study products in total fibre between the control and HBG group only accounted for 92-94% of the total fibre differences.

The only dietary variation between groups was the monounsaturated fats which varied at baseline also. The levels were not different at mid-point and 3 months but rather the change from baseline produced an interaction effect for time x group (Table 5.5). Therefore, all anthropometric and biochemical results were also checked for correlation with the monounsaturated fat levels.

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 other results.

**Table 5-7 Average of mid and final results for contribution of  $\beta$ -glucan and total fibre by study products.**

	<i>Group</i>	<i>Beta- Glucan (fibre in grams)</i>		<i>Total (fibre in grams)</i>	
		<i>mid-pt</i>	<i>final</i>	<i>mid-pt</i>	<i>final</i>
Contribution of study products to fibre intake	control	0	0	6.3	6.1
	MBG	5.7	5.0	11.8	11.7
	HBG	7.0	7.6	15.5	16.6
Total Fibre from food records	control			24.2	21.8
	MBG			28.0	27.4
	HBG			34.2	33.0
Likely contribution to fibre difference from $\beta$ -glucan in products compared to control		Control			
		MBG		100%	89%
		HBG		70%	67%
Likely contribution to fibre difference from total fibre in products compared to control		Control		-	-
		MBG		>100%	100%
		HBG		92%	94%

\* subjects were not actively discouraged from consumption of oat products, but cereal compliance was high so minimal levels of  $\beta$ -glucan are likely to be consumed from snack products currently available commercially.

### 5.3.3 Clinical Indices

Almost all anthropometric and biochemical indices changed over time although there were no significant differences between the groups for any measured clinical parameter over time (Table 5.8). The average weight loss for the study sample of 4.1kg was significant ( $P<0.001$ ) (ranging from a 0.2kg gain to a 14.9 kg loss). However, this is less than the anticipated 6-6.5 kg designed for the energy restricted diet. It is also less than the change in kilojoule intake demonstrated in the food records would predict. The weight loss was not significantly different between groups ( $P=0.921$ ). Waist measurements decreased significantly ( $P<0.001$ ). Malfunctioning bioimpedance equipment meant results for the percentage body fat could not be used.

Fasting blood glucose results did not decrease however, fasting insulin decreased significantly compared to baseline ( $P<0.001$ ). However, no differences between groups were identified ( $P=0.184$ ) (Table 5.8). Log transformation of insulin data showed the difference from baseline to 3 months was still significant ( $P=0.001$ ) but not related to the intervention ( $P=0.113$ ).



Regression analysis indicated that weight loss significantly predicted waist change ( $P=0.001$ ), leptin ( $P=0.007$ ), BGL ( $P=0.031$ ) and insulin ( $P=0.052$ ). However the predictions, other than the expected waist change ( $R^2=0.480$ ) were relatively weak ( $R^2=0.130, 0.084$  and  $0.069$  respectively). Adjusting changes in these parameters for weight loss, failed to identify any significant differences between the groups.

Lipid results were examined overall, between groups and also reviewing data for those subjects who had elevated lipids at baseline. No variation in decreased cholesterol was demonstrated between the groups, with mean decreases of  $0.19$  (SD  $0.79$ ),  $0.31$  (SD  $0.55$ ) and  $0.56$  (SD  $0.61$ ) mmol/L for the control, MBG and HBG respectively ( $P=0.239$ ). The general trend was as expected with the greatest decrease in cholesterol with the highest dose of  $\beta$ -glucan. Overall, there was a significant decrease in cholesterol over three months ( $P<0.001$ ). Reviewing data for subjects with mildly elevated cholesterol ( $>4.0$  mmol/L) or true hypercholesterolaemia ( $>5.5$  mmol/L) did not alter these results, although there was still no significant difference between the groups. Mean decreases in cholesterol for subjects with baseline above  $4.0$  mmol/L were  $0.47$  (SD  $0.56$ ),  $0.13$  (SD  $0.45$ ) and  $0.52$  (SD  $0.63$ ) mmol/L for control, MBG and HBG respectively.

For all subjects, HDL levels significantly decreased over time ( $P<0.001$ ), LDL significantly decreased ( $P=0.028$ ) and triglycerides did not change ( $P=0.353$ ). Although the LDL results for RMANOVA did not show an overall effect between groups, post hoc Bonferroni adjustments indicated a trend to difference between the control and high dose of  $\beta$ -glucan ( $P=0.077$ ) with the greatest decrease in LDL in the HBG group (Table 8). No significant ( $P<0.05$ ) differences were detected between the groups for the other lipid parameters.

**Table 5-8 Changes in Clinical indices overtime and between control, MBG and HBG groups.**

<i>Changes in Clinical indices</i>	<i>All Groups</i>			<i>Control</i>		<i>MBG</i>		<i>HBG</i>		<i>P value<sup>b</sup></i>
	<i>Baseline Data</i>	<i>Mean Change</i>	<i>P value<sup>a</sup></i>	<i>Baseline</i>	<i>Mean Change</i>	<i>Baseline</i>	<i>Mean Change</i>	<i>Baseline</i>	<i>Mean Change</i>	
Weight (kg)	78.4	-4.1 ± 3.1	<0.001	77.6 ± 7.8	4.0 ± 2.4	80.9 ± 8.23	-4.3 ± 3.7	76.6 ± 6.5	-3.9 ± 2.9	0.921
Waist (cm)	83.8	-4.1 ± 2.8	<0.001	83.8 ± 5.7	-4.0 ± 2.6	85.3 ± 7.9	-0.8 ± 3.16	82.0 ± 5.5	-3.4 ± 2.5	0.476
Glucose(mmol/L)	4.72	0.02 ± 0.47	0.586	4.75 ± 0.57	-0.01 ± 0.41	4.89 ± 0.46	0.03 ± 0.43	4.52 ± 0.37	0.15 ± 0.55	0.420
Insulin (mmol/L)	11.37	-2.68 ± 3.75	<0.001	10.74 ± 4.29	-3.16 ± 4.59	10.92 ± 5.70	-1.42 ± 3.11	12.40 ± 4.83	-3.40 ± 3.50	0.184
Chol. (mmol/L)	5.03	-0.37 ± 0.65	<0.001	5.21 ± 0.73	-0.19 ± 0.79	5.02 ± 1.07	-0.31 ± 0.55	4.91 ± 1.30	-0.56 ± 0.61	0.239
HDL (mmol/L)	2.91	-0.23 ± 0.2	<0.001	1.59 ± 0.38	0.24 ± 0.10	1.64 ± 0.27	-0.16 ± 0.19	1.70 ± 0.33	-0.3 ± 0.25	0.091
LDL (mmol/L)	1.65	-0.14 ± 0.48	0.028	3.23 ± 0.47	-0.13 ± 0.54	2.89 ± 0.99	-0.08 ± 0.50	2.67 ± 0.92	-0.23 ± 0.42	0.692
TG (mmol/L)	1.11	-0.09 ± 0.43	0.353	1.27 ± 0.40	-0.05 ± 0.29	1.06 ± 0.49	-0.13 ± 0.26	1.03 ± 0.98	-0.01 ± 0.72	0.688
Ghrelin (pg/mL)	53.4	-6.4 ± 29.5	0.217	45.4 ± 21.0	-6.9 ± 18.7	65.0 ± 72.9	-9.3 ± 33.9	47.2 ± 29.6	0.0 ± 33.8	0.957
Leptin (pg/mL)	20621	-7133±5720	<0.001	18689±9338	-7054±6474	20300±9163	-5343±4819	22605±6514	-9429±5390	0.078
GLP (pg/mL)	47.95	-6.9 ± 9.3	<0.001	49.6 ± 16.7	-7.7 ± 9.5	50.3 ± 15.0	-8.0 ± 8.9	43.8 ± 14.6	-5.0 ± 9.7	0.493
PYY (pg/mL)	85.80	-12.0 ± 26.2	0.015	92.4 ± 32.1	-5.6 ± 11.8	89.1 ± 19.7	-23.4 ± 31.5*	77.1 ± 32.9	-1.3 ± 19.6*	0.018
PYY336 (pg/mL)	73.23	6.1 ± 21.0	0.195	71.4 ± 11.7	2.5 ± 18.2	71.0 ± 19.8	10.1 ± 18.4	77.3 ± 25.1	8.9 ± 25.8	0.807
CCK (pg/mL)**	218	177 ± 170	<0.001	186 ± 167	219 ± 147	245 ± 159	139 ± 171	210 ± 171	186 ± 187	0.253

<sup>a</sup> P values for change from 0 to 3 months    <sup>b</sup> P values for RMANOVA changes between groups

\* P≤0.05 when compared to control using Bonferroni adjustments. No significant difference existed between MBG and HBG.

\*\* Results for CCK were higher than usually measured. Peptides were not extracted prior to analysis and this may have affected absolute results, however as this is a repeated measures analysis trends can still be detected if they exist.

Large standard deviations existed within all the data sets limiting the significance of results, especially hormonal changes. The decrease in leptin levels for the study sample was significant over time ( $P < 0.001$ ) and regression analysis indicated the association with decreasing body weight ( $P = 0.007$ ). Between groups, the differences approached significance ( $P = 0.078$ ) with the greatest decrease in leptin identified in the HBG group, even with the same body weight change.

Total PYY levels decreased significantly over time (RMANOVA,  $P = 0.015$ ) and this was significantly different between groups ( $P = 0.041$ ). Post-hoc Bonferroni adjustments indicated that the control group produced significantly different effects compared to both the MBG ( $P = 0.021$ ) and HBG ( $P = 0.050$ ) groups where the smallest decrease in PYY was seen in the HBG group. The greatest change was in the MBG group so this would not seem related to dose of  $\beta$ -glucan. Even though levels of total PYY decreased in all groups those for PYY<sub>3-36</sub> increased, although the latter was not statistically significant ( $P = 0.195$ ). PYY<sub>3-36</sub> levels increased more with the inclusion of  $\beta$ -glucan in the diet (8-10pg/mL increase compared with 2.5 pg/mL in the control) but this was not significant between groups ( $P = 0.807$ ).

There was a significant decrease in GLP-1 over time ( $P < 0.001$ ) but no differences between groups ( $P = 0.567$ ). Ghrelin levels did not significantly alter over time ( $P = 0.217$ ) and there were no between group effects ( $P = 0.632$ ). CCK results seemed elevated most likely due to a lack of extraction of peptides however, as this was a repeated-measures analysis, results were included. CCK levels increased significantly over time ( $P < 0.001$ ) but there was no group effect ( $P = 0.969$ ) with all groups increasing CCK levels at equal rates.

All variables were reviewed adjusting for weight loss and monounsaturated fat, but other than the correlations described previously, no significant trends were noted. The large standard deviations indicated that individual fluctuations are more varied than any overall effect of increased  $\beta$ -glucan.

#### 5.3.4 Subjective satiety

VAS results were reviewed for individual time points to compare any differences which may have existed between the control and test groups (ANOVA). No significant

differences were identified between the groups at any time point tested – that is all individuals showed similar hunger/fullness at the same test points. Table 5.9 details VAS results with P values for one-way ANOVA.

### 5.3.5 Product evaluation

The majority of subjects in the intervention groups reported some negative physical symptoms from the products (52% for MBG and 63% for HBG) although this was less than the numbers experiencing positive symptoms. Negative symptoms included abdominal pain (4/19 HBG subjects), diarrhoea (1/16 control, 2/21 MBG, 1/19 HBG subjects) and constipation (2/16 control, 1/21 MBG, 3/19 HBG subjects). Although the numbers of symptoms were high only one subject in the entire study described these as severe and significant enough to modify intake. This subject was in the HBG group and continued the cereal and one snack daily rather than two.

In addition to recording of negative symptoms, subjects were asked to detail if they believed they had experienced positive symptoms as a result of consuming the study products. Forty-seven of fifty-six subjects believed they had experienced positive symptoms (16/16 controls, 14/21 MBG and 17/19 HBG subjects). Positive associations with the study products included “feeling more full for longer”, “healthy snacking avoiding unhealthy snacking”, “less peaks and lows” in intake, “more regular bowel movements” and “increased energy”. (Full evaluation is detailed in Appendix 5-2)

**Table 5-9 Visual analogue scale P values for one way ANOVA**

		<i>Before breakfast</i>	<i>2 hours post- breakfast</i>	<i>Before lunch</i>	<i>2 hours post- lunch</i>	<i>Before dinner</i>	<i>2 hours post- dinner</i>
<i>How hungry are you now?</i>	zero	0.103	0.827	0.754	0.558	0.281	0.927
<i>How satisfied are you now?</i>	zero	0.163	0.193	0.599	0.187	0.641	0.722
<i>How full do you feel?</i>	zero	0.127	0.664	0.295	0.220	0.765	0.729
<i>How much could you eat?</i>	zero	0.363	0.631	0.190	0.236	0.320	0.437
<i>How hungry are you now?</i>	mid-pt	0.994	0.370	0.077	0.986	0.968	0.397
<i>How satisfied are you now?</i>	mid-pt	0.804	0.166	0.518	0.680	0.838	0.571
<i>How full do you feel?</i>	mid-pt	0.898	0.843	0.445	0.996	0.697	0.501
<i>How much could you eat?</i>	mid-pt	0.979	0.693	0.889	0.882	0.772	0.273
<i>How hungry are you now?</i>	final	0.992	0.896	0.488	0.701	0.734	0.648
<i>How satisfied are you now?</i>	final	0.471	0.364	0.818	0.706	0.934	0.558
<i>How full do you feel?</i>	final	0.932	0.218	0.922	0.431	0.829	0.735
<i>How much could you eat?</i>	final	0.938	0.867	0.958	0.661	0.881	0.453

The products were also evaluated from an organoleptic perspective using questionnaires with likert scales. The full results are detailed in Appendix 5-4. Overall, all the RTE cereals received favourable evaluations. The porridges overall were disliked and consumed at a minimal level. Muesli bars were well received but the HBG cereal snacks taken in the afternoon were less well liked than the low fibre alternative. That is, subjects in the MBG group who could compare would choose the HBG muesli bar and the control snack to make up their choices most often.

## 5.4 Discussion

Within the context of this study, regardless of the diet subjects were on, they lost weight, with the expected changes in waist circumference, fasting insulin<sup>205</sup> and lipids<sup>206</sup>, and leptin<sup>207</sup>. Even though weight loss was modest, the change of 5 per cent if maintained even at 3-4 per cent would most likely decrease incidence of development of type 2 diabetes in this moderately overweight, but at risk group<sup>208</sup>. The waist circumference of the group as a whole decreased significantly and as a measure of abdominal adiposity indicates decreased risk of insulin resistance, glucose intolerance and dyslipidaemias<sup>209, 210</sup>. The fact that the subjects were only moderately overweight with limited progression towards metabolic syndrome, most likely limited the ability of the study to differentiate subtle differences that may have existed between groups. Nevertheless, energy restriction alone remained effective.

Much of the research with  $\beta$ -glucan as a functional ingredient focuses on improvements in parameters such as glycaemic control and hypercholesterolaemia. The research here used healthy subjects whose “healthy overweight” status ensured some elevated lipids but minimal insulin resistance. Greater effects may have been seen in a population with diabetes with greater metabolic dysfunction. The change in cholesterol, greatest for the HBG group may have been more exposed with more subjects, but this was a secondary outcome measure. Literature on non-hypercholesterolaemic or mildly hypercholesterolaemic individuals tends to show limited response to  $\beta$ -glucan, so the borderline results would be expected, particularly given the observed weight loss. The greater than 10% decrease in the HBG group provides support for oat  $\beta$ -glucan as a dietary intervention agent in the management of hypercholesterolaemia. The observed decrease in HDL levels was unexpected, but the reported consumption of saturated fats, was notably higher than polyunsaturated fats (Table 5.4), so the background diet may not have been favourable.

With the modest weight loss demonstrated here there were still significant changes in PYY and GLP-1. Additionally there was an overall decrease in PYY while maintaining or perhaps increasing the fractions involved with satiety PYY<sub>3-36</sub>. It has been postulated that PYY has a role in the aetiology of human obesity due to negative correlation with BMI and the fact that injection of the active fraction of PYY, PYY<sub>3-36</sub> decreases food intake in humans<sup>126</sup>. PYY is secreted in the same gut endocrine cells as

GLP-1, both inhibit gastric emptying and promote satiety, and are released in response to food. GLP-1 having an additional or additive role in its regulation of blood glucose via increasing insulin sensitivity <sup>132</sup>.

The observed relationship between changes in hormones and weight loss was perplexing. Nevertheless, recent research has shown that with a very large weight loss, as seen in surgical interventions for obesity, fasting levels of PYY increases but GLP-1 decreases, suggesting a less easily defined relationship between the two hormones and that they are not co-dependent <sup>211</sup>. Studies using hypocaloric diets to induce weight loss have identified a decrease in GLP-1 response to dietary stimulus with weight loss <sup>212</sup>.

In clinical trials fasting PYY and often PYY<sub>3-36</sub> have been shown to be decreased in obesity. Roth identified decreased PYY<sub>3-36</sub> in obese children which increased with weight loss and showed that this increase was predictive of successful weight maintenance <sup>127</sup>. Pfluger et al found no difference in fasting PYY<sub>3-36</sub> between lean and obese subjects, however identified a 30% decrease in fasting total PYY when obese subjects lost approximately 5% of their body weight <sup>122</sup>. The results from the current study broadly mimic these results with an overall decrease in PYY but an increase or at least maintenance in PYY<sub>3-36</sub>. Animal studies in our laboratory <sup>196</sup> have indicated an increase in PYY<sub>3-36</sub> with increasing doses of  $\beta$ -glucan and this together with the trend in this study warrant further investigation.

The group effect noted in fasting PYY, where both MBG and HBG results are different to the control, is difficult to interpret, given the smallest decrease was in the HBG and the largest decrease in the MBG. Other results which show similar trends (MBG a greater difference in one direction compared to the control or HBG such as with leptin) do not show significant correlations with the PYY results so it is difficult to infer a mechanism based on a U-shaped curve, where a certain dose would produce negative results but a greater dose would achieve desired outcomes. For example, in the current study no correlations existed with monounsaturated fat intake, which was highest in the MBG group, so the differences may just be statistical aberrations, in part created by the large variations in responses. Similarly, the larger change in leptin in the HBG group with the same weight loss seems positive but the smallest change was with the MBG group and no dietary correlations exist.

We found a high overall acceptance of the high doses of fibre and good maintenance of the solubility, molecular weight and viscosity of the  $\beta$ -glucan after. No differences were identified with the subjective measures of satiety. The use of visual analogue scales is most likely to be accurate in a controlled situation such as in acute meal test studies in a laboratory situation <sup>157</sup>. So it is perhaps not surprising that small differences from month to month are unlikely to be quantified by subjects using the scales.

Despite attention to product attributes that theoretically affect satiety and tight dietary controls, this study failed to confirm direct effects of set levels of  $\beta$ -glucan on weight loss in an energy-restricted diet. Epidemiological evidence strongly suggests that high fibre diets have positive effects on weight control <sup>125</sup>. Separating out the effects of a single fibre remains a difficult task within a human intervention trial and it would seem the major outcomes in this study are similar to another recent intervention trial with soluble fibre <sup>213</sup> which also could not find an intervention effect with respect to weight loss. The lack of positive results such as these could certainly be used to support the notion that “fibre” is not just an indigestible ingredient in a food but is a part of healthy diet which includes grains, fruits and vegetables perpetuating good health. However, there are a number of confounding variables in this study which warrant consideration.

Firstly, the current trial was only of three months duration. Studies identifying positive effects of fibre, such as decreased weight gain usually last for a number of years <sup>45</sup>. A 3-month intervention may not be long enough to separate out the differences between the subjects’ desire to comply and the actual effects of the dietary intervention. This difference is more likely to be obvious if subjects were to follow a particular eating pattern over a longer timeframe. In a recent study, researchers found that regardless of the dietary intervention, subjects who reported greater compliance with a weight loss regimen lost a greater amount of weight <sup>214</sup>. The authors conclude that strategies to increase adherence may be more important than dietary composition.

Secondly, this study included products for the control group which were still relatively high in fibre. Only the amounts of  $\beta$ -glucan varied within the diet. It seems likely that the effect of any one ingredient, even if positive, will be relatively small and hence showing a statistical difference between groups will be difficult, especially over only three months. The numbers of subjects identified as necessary to detect a difference in this study was based on work of Solum in 1987 <sup>202</sup> who used an energy restricted diet



with overweight women to determine the possible benefits of fibre supplementation. Control subjects used placebo tablets while the intervention group had approximately 6g of fibre sourced from grains and citrus fruit fibres. Over 12 weeks the intervention group lost 1.8 kg more. The way subjects consumed the fibre in the Solum study (capsules with water just before each meal) may have affected the outcomes. The overall greater weight loss 6.7 and 8.5 kg for the control and intervention groups respectively, in this supplement study shows that adding food items to a dietary regimen (as in the currently reported study) may decrease weight loss to some degree. However, insisting subjects ate particular breakfast and afternoon tea items may have increased caloric consumption at times when subjects may have chosen to eat less in a non-directed environment.

Finally, it is difficult to control the intake of human subjects. Although food records and subjective discussions with subjects indicated high compliance with consumption of study products overall, subjects were less compliant with the energy restriction as evidenced by the modest weight loss. The greatest number of subjects withdrew from the control group, which could infer the greatest difficulty with compliance, but this is unable to be confirmed within the constraints of the ethical requirements of the intervention trial.

In summary, although the groups experienced general improvement in measures such as fasting insulin and cholesterol, associated overall with weight loss, no effects seem specifically related to  $\beta$ -glucan dose. Most likely, some differences would have been realised if the study continued in the longer term, as generally, subjects with higher fibre will maintain weight more easily. However, within the timeframes of an intervention trial, and realistically, the time frames individuals may pursue a “weight loss diet”, there were no discernable differences between a regular energy-restricted diet and that which contained significant  $\beta$ -glucan.

**CHAPTER 6      DEFINITIONS OF FIBRE AND PROPOSAL 293 –  
EFFECTS ON FOOD LABELLING**

## 6.1 Introduction

The introductory chapter to this thesis discusses fibre definitions. This chapter reviews how these definitions may affect claims of food packaging together with changes to food regulation in Australia which may also affect this labelling. Dietary fibre definitions are contentious in part because the types of food which contain fibre may have multiple, beneficial constituents (such as fruit which has fibre and antioxidants) but also because early analytical techniques of measurement focussed on fibre as “naturally occurring” and “non-digestible” and in fact the former does not have to be true and the latter is difficult to measure within a physiological system.

Hence definitions of dietary fibre, such as that proposed by the US Food and Nutrition Board <sup>20</sup> which describe endogenous fibre as dietary fibre, together with functional fibre as fibres which have beneficial physiological effects in humans, attempt to separate fibre sources but also possible health benefits. However, it would not be possible to separate physiological effects caused by multiple fibre sources within a single food and the ingredient listing, while describing the origin of the fibre (by this definition) could not ascribe separate health effects if the food was tested as a whole. Defining allowable physiological effects of fibre will be just as difficult as assigning a definition and will require significant effort from scientists and regulators <sup>21</sup>.

## 6.2 Regulation of Food Labelling in Australia: Relationships to fibre definitions

To determine what impact changing definitions and changing regulation have on consumers, it is important to consider why nutrient information was included in the first instance. The mission of FSANZ is to provide a safe food supply and have well informed consumers <sup>215</sup>. Included in its overview is information aimed at maintenance of broad community support and public confidence in regulatory decisions through maintenance of collaborative arrangements between all levels from regulators and primary producers through to consumers <sup>215</sup>.

Generally, FSANZ regulates diet and health claims including diet and disease claims, along with nutrient function claims <sup>29</sup>. The inclusion of content claims in regulation recognises that ingredients often infer health benefits regardless of the specific food,

ingredient source and precise function of that nutrient. Yet consumers are unlikely to recognise very specific notions related to dietary fibre, such as calculations of energy values, unless it is part of current nutrition education <sup>216</sup>. Similarly individual consumers are unlikely to recognise and differentiate types of fibre and sources.

Alterations in definitions of fibre (and analytical methods) will affect both nutrient information panels (NIPs) and nutrient databases. Typically, current NIPs in Australia may list both total fibre and sometimes soluble fibre. Nutrient databases are mostly less specific and only list total fibre contents. However, if definitions change it is possible that “fibre” may become dietary fibre and functional fibre. So a food with endogenous fibre as well as added extracted or purified fibre for a health benefit (such as the addition of soluble fibre to cereals for cholesterol lowering benefits), would need to differentiate fibre ingredients even if the two fibres originated from the same grain source.

Obviously such complexities would require review before the definitions could be adopted for use with consumers. If a food is good for laxation and for serum lipid control, are the precise percentages always relevant, or are they only most relevant to health professionals or researchers looking to magnify effects? A single food may produce only small effects in isolation but combined with other dietary guidelines towards the same purpose, it may produce a positive outcome. Isolating single effects is often difficult but is pursued in part because consumers would like a “magic bullet” solution, but also because this effect, if magnified, may provide significant commercial outcomes.

Front of package labelling is less likely to change due to changes in definitions unless the most strict of definitions is applied to dietary fibre, as this labelling tends to quantify total amounts of fibre. However, these claims are likely to change under P293. Currently, food standards classification allows nutrient claims for “a source of fibre” (S1), “a good source of fibre” (GS1) and “an excellent source of fibre” (ES1). This classification would change based on proposed changes to regulation (P293) with a source (S2), good source (GS2) and excellent source (ES2) of fibre representing greater amounts of fibre contained in each serve (Table 6.1). Serves sizes are defined by food manufacturers and so changes relating to fibre per serve may tempt some manufacturers to alter serve sizes in order to maintain classifications as sources of fibre.

**Table 6-1 Grams of fibre required for front of packet labelling of fibre sources.**

<i>Labelling of Fibre Claims</i>	<i>Grams of fibre current legislation</i>	<i>Grams of fibre proposed in P293</i>
Source	1.5	2.0
Good Source	3.0	4.0
Excellent Source	6.0	7.0

### **6.3 Food Standards regulation: Insights from the current research**

To demonstrate the impact changes proposed in P293 may have on what manufacturers are able to call a source of fibre a pilot study based on baseline dietary records from a 3-month weight reduction intervention trial (Chapter 5) were reviewed using current and proposed guidelines. Subjects were all female with BMI range 25-35 kg m<sup>-2</sup>. The quantity of foods consumed was assessed from three-day food records collected from 48 subjects as a sample of meal patterns in this single population group. The differences which proposed changes to P293 would make to dietary fibre source claims on foods consumed were assessed.

Analysis was limited to foods within the breads and cereals food group. Serve sizes were based on information from manufacturers. Where precise details were absent, serves were matched for approximate carbohydrate values to the most common bread or cereal products. The bread serve size was 2 slices, and the pasta serve one cup. Breakfast cereal serve sizes were 30-45g depending on the type of cereal. The total number of serves of breads and cereals were calculated and subdivided according to current food standards classification S1, GS1 and ES1. This classification was then re-calculated based on proposed changes - S2, GS2 and ES2 (Table 6.2).

The number of serves in each category under current and proposed regulation were compared using students' t-test to determine significant differences. Qualitative analysis of the types of foods included in each fibre category, in particular those which would need to change packaging content claims was also undertaken.

**Table 6-2 Comparisons of number of serves of breads and cereals from dietary records (N=48), classified as sources, good sources and excellent sources of fibre using current and proposed (P293) regulation.**

<i>Fibre classification</i>	<i>No. of serves - current <math>\pm</math> SD</i>	<i>No. of serves – P293 <math>\pm</math> SD</i>	<i>t-value for comparisons</i>
source	1.25 $\pm$ 0.98	1.30 $\pm$ 0.76	0.699 (S1 vs S2)
good source	1.06 $\pm$ 0.80	0.72 $\pm$ 0.76	<0.001 (GS1 vs GS2)
excellent source	0.19 $\pm$ 0.51	0.07 $\pm$ 0.32	0.038 (ES1 vs ES2)

Subjects averaged a moderate  $3.4 \pm 1.1$  serves of breads and cereals each day (range of 1.0-7.2). The data showed significant decreases ( $P < 0.001$ ) in the number of foods which could be labelled as “good sources” of fibre if P293 changes are accepted. These related to products containing between 3 and 4 grams of fibre, which would no longer be labelled as good sources but rather became ‘sources’ of fibre. The main food products responsible for this decrease were fibre-increased white bread (2 large slices) and cereals such as wheat breakfast biscuits, where 2 biscuits is a common serve. Products such as wholemeal muffins, and certain grain breads, pastas and crispbreads would also no longer be eligible to claim “a good source of fibre”.

The main product affected by changes in the “source of fibre” classification was white bread which tended to contain just over 1.5 g of fibre per two slices, but less than the new regulation of 2 g. The number of foods carrying a label of “source of fibre” would not change according to this analysis ( $P = 0.699$ , current vs proposed fibre levels) as although some foods could no longer make the claim, the foods no longer able to claim “good source” that would become “source of fibre” compensated for this change. The change in excellent fibre sources is minimal although statistically significant. This is relevant in food terms because at present, cereals such as muesli (with between 6 and 7 grams of fibre) currently attract the “excellent” claim. Under the new position, without any other changes they would become a “good source” of fibre.

#### **6.4 Labelling complexities**

If food labelling must differentiate between fibre sources, does this benefit the consumer? It is generally accepted that consumers want more information about their food and also on how to read food labels. When does the added complexity of a label negate the advantage of the information given? The answer is most likely within the

scientific research food manufacturers and institutions conduct about functional ingredients. If an ingredient is shown to have the same effect when added to a food, as it has in the food from which it is originally sourced, then there can be little perceived benefit in separating ingredient sources. For example, viscous, high molecular weight  $\beta$ -glucan has been associated with cholesterol lowering in hypercholesterolaemic individuals<sup>80</sup>. It does not matter whether directly from oats, or from other products with added concentrated  $\beta$ -glucan, as long as the viscosity and solubility are maintained<sup>107</sup>. Therefore, the precise definition given to the fibre – dietary or functional - is less important than the actual functionality.

Similarly, definitions that state fibre is only “intrinsic plant cell wall polysaccharides” undervalue the functionality of the modern food supply. Critical to labelling information is the amount of fibre and its functionality, rather than whether it is added or endogenous. Although it is contentious as to whether or not added fibre is as functional as that which is naturally occurring, the onus of proof lies with scientists and commercial parties to identify the functionality whether it lies with the ingredient, a specific component of the ingredient, the food or a food system.

## 6.5 Labelling for food professionals

Definitional and labelling information is of significant importance to healthcare professionals, in particular dietitians, who rely on label information and nutrient databases, to calculate therapeutic diets or to direct consumers. Changes in definitions or calculations of fibre affect these professionals as they are more likely to require precise information on the ingredient source as well as its functionality. Any change in definitions of fibre must be clearly communicated to this group, as fibre values may change with these definitions, but that does not mean a food has actually changed.

Finally, food manufacturers are affected by definitional changes described in that the categorisation of ingredients may change. By some definitions, the term fibre would be split into dietary and functional fibre as opposed to terms such as soluble and insoluble fibre. Proponents of definitions related to functional definitions of fibre recognise variations within the categories of soluble and insoluble fibre and this is the reason to remove this labelling from foods. Ideally manufacturers would label total fibre, and if necessary, define a number of grams of a specific fibre type which could be associated with a specific function.

Changes to content claims relating to fibre, as described in P293, affect some key common foods and it remains to be seen whether or not manufacturers add extra fibre to some products where functional fibre is already added (eg white fibre increased breads) or even attempt to alter serve sizes in lower fibre foods (eg regular white bread). It is of interest whether or not changing of labelling of foods such as wheat breakfast biscuits, previously labelled “a good source of fibre” and now “a source of fibre”, changes any purchasing practices. Most likely, the association consumers have with fibre and health would not change at the omission of a single word.

Evidence as to whether purchasing or consumption patterns would change with increased requirements for fibre source labelling appears one of the major contentions of those opposing the proposed changes<sup>217</sup>. The proposal from Dietitians’ Association of Australia argued against the changes in P293 based on these grounds. It is of note that some items such as a half cup serve of certain vegetables would no longer warrant a claim. However, given that currently white bread is eligible for a content claim (S1) one could argue it is better to direct consumers toward higher fibre items to actually



increase fibre consumption. The original levels of fibre were set to deliberately include white bread <sup>218</sup> while these new guidelines more broadly parallel guidelines such as those for protein, where 5 g (10% of the reference value for protein) is a “source” of protein <sup>27</sup>.

## **6.6 Conclusions**

With all functional ingredients there will be arguments against their use, particularly when the ingredient can be consumed in its endogenous form. However, not all foods are consumed in a strictly endogenous state and it is unrealistic to expect manufacturers not to aim to improve foods in order to make a claim. Provided a functional ingredient is proven to have an effect in the food it is added to, it should not matter where it came from provided general ethical principles are upheld. Definitions must be reflective of the component or ingredient, be able to be measured accurately and consistently, and in the modern age of consumer knowledge seeking, they must correctly describe a function. Definitions which only meet some of these caveats are not useful for consumers, scientists or health professionals. Work on methods to describe total fibre should be applauded, as should any projects which investigate food matrices and functional ingredients, including fibre.

## **CHAPTER 7      CONCLUSION**

**Evidence for effects of oat  $\beta$ -glucan on satiety and weight control**

## 7.1 Hypothesis

Combining the research within this thesis with the body of evidence in the literature there is evidence that  $\beta$ -glucan promotes satiety. There is an increase in anorexigenic hormones after ingestion of  $\beta$ -glucan in a dose responsive manner. Subjective satiety is increased after relatively small doses of  $\beta$ -glucan and trends are seen towards a decreased dietary intake at a subsequent meal after consumption of a dose greater than 5g. These findings did not translate into expected outcomes in the 3-month dietary intervention trial to result in greater weight loss for those subjects consuming  $\beta$ -glucan, compared to a control consuming moderately high fibre without  $\beta$ -glucan. Currently there is insufficient evidence to support a health claim related to weight control.

## 7.2 Research Design and Evidence Requirements

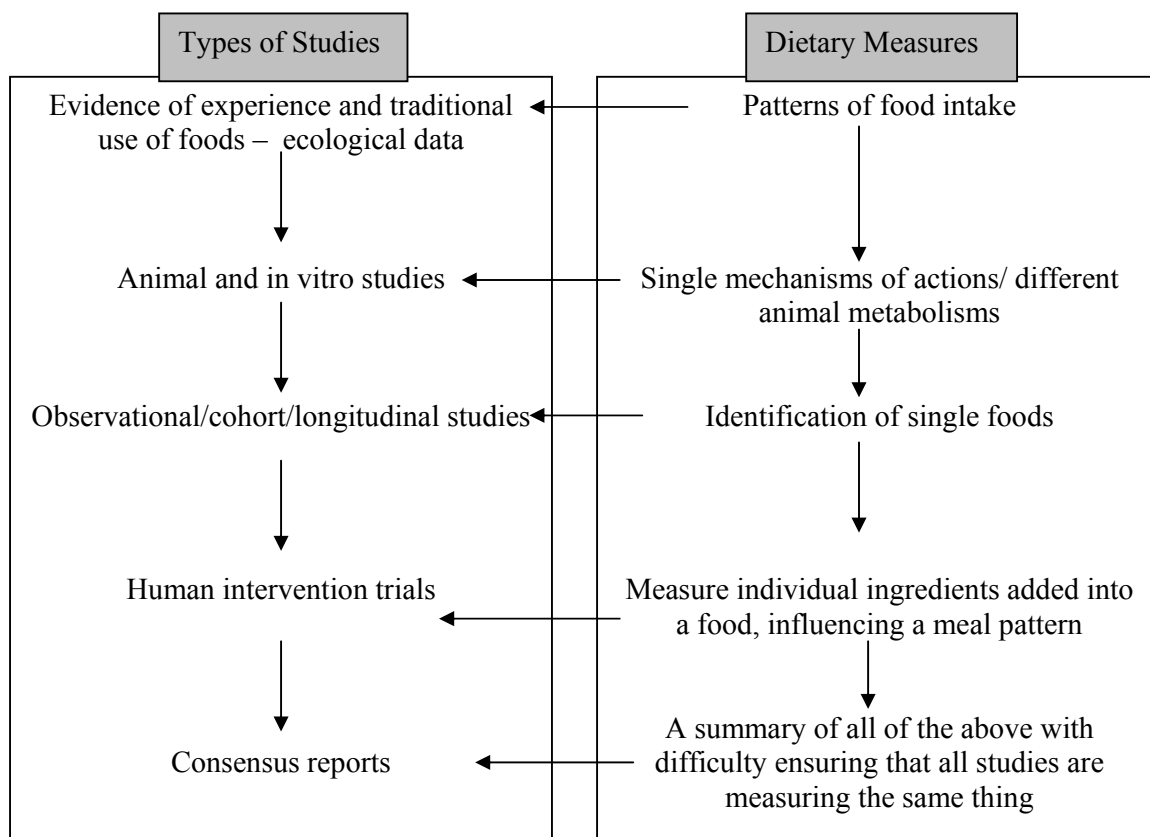
Science-based research into the effects of a food component, ingredient or whole food adheres to the general principles for scientific substantiation where the methodologies and design are well documented and the outcomes independently reviewed and conclusive. However, in addition there are specific requirements suggested by world-wide food regulatory bodies, summarised well by the European Food Safety Authority<sup>219</sup>:-

- the claimed effect of the food/constituent is relevant for human health,
- a cause and effect relationship is established between the consumption of the food/constituent and the claimed effect in humans (such as: the strength, consistency, specificity, dose-response, and biological plausibility of the relationship),
- the quantity of the food/constituent and pattern of consumption required to obtain the claimed effect could reasonably be achieved as part of a balanced diet,
- the specific study group(s) in which the evidence was obtained is representative of the target population for which the claim is intended.

A hierarchy of evidence in relation to substantiation of health claims on foods would dictate that we review evidence as described in the left-hand box of Figure 7.1 below.

However, the reality is that as we move up the hierarchy to intervention trials, we may move away from a pattern of eating which directed us towards the health benefits initially (right-hand box of Figure 7.1).

The progression from ecological observations to an intervention trial means we move not only from established traditional patterns of eating, but in adding a product to an existing meal plan, we change that meal plan. This means there is potential to lose any synergies that may have existed in the original eating pattern. In addition there are changes that may take place in an ingredient or component thereof, when we extract, concentrate, process and prepare it for consumption. All of these scientific considerations sit in a web of legislation which exists around definitions and debates on what is being measured (for example, debates about fibres and wholegrains) and precisely how that claim will be presented to consumers.



**Figure 7-1 Levels of evidence compared with measured food intake patterns**

Although this sounds very negative, as if a health claim cannot be proven, it is actually more of a description as to why in development of evidence for health claims, the elements listed below and discussed with reference to  $\beta$ -glucan, satiety and weight control, are a minimum standard for research methodologies likely to support a health claim.

1. *Clear elucidation of the ingredient/functional component.* In the case of  $\beta$ -glucan this includes measurement of MW, viscosity, solubility and concentration and recognition that, different sources of  $\beta$ -glucan (from different grains or foods; whether concentrated or extracted) may have slightly different micro-environments and therefore the source should be clearly defined. In addition, in most concentrated sources of  $\beta$ -glucan it is not entirely possible to separate  $\beta$ -glucan effects from those of the other components such as insoluble fibre.
2. *Precise measurement of “the component” in the diet (whether cohort or intervention) including useful markers of consumption.* This should include measurements of all of the nutritional components of the product as well as recording of consumption and measurement of unused samples in an intervention. Most importantly, the components measured must meet the definitions suggested by regulatory bodies. In the case of  $\beta$ -glucan it is a measure of all possible sources of  $\beta$ -glucan as a source of fibre, together with other sources of soluble fibres which may have similar effects. Blood markers, such as those available for omega-3 fats are not yet available for cereal fibres.
3. *Changes in an eating pattern as a result of inclusion of the functional component/ ingredient in the meal pattern.* Wherever possible use of three-day food records or multiple, random 24-hour recalls both before, during and after intervention will measure changes in the entire diet as a result of the intervention which must be considered in any interpretation of results.
4. *Useful markers of outcome measures or actual outcome measures which are recognised in relation to the health benefit described.* In relation to overweight and obesity, the actual key outcomes are weight loss or decreased body weight gain. In addition, identifying decreased food intake is a genuine marker of this, while measures relating to satiety and appetite, including hormones are only

markers, as they provide an insight into mechanisms of action. There must always be recognition that subjective satiety, dependent or independent of these hormone markers, is not always linked to decreased dietary intake and subsequent weight loss.

5. *Consumption over realistic time frames to demonstrate an effect based on the proposed mechanism.* Meal test studies will mostly only show mechanisms in relation to satiety, although measurement of subsequent meal intake or total intake over the day is supportive of decreased intake as a possible mechanism for weight loss. This is the minimum time-frame for gathering of results. An effect related to weight control or weight loss ideally would be followed for greater than 3 months and ideally 12-24 months.
6. *Knowledge of mechanism may also inform other factors which may negate/enhance effects.* Knowledge of hormone mechanisms for decreased appetite identifies markers in relation to hunger and satiety. Identifying these in relation to  $\beta$ -glucan may give evidence of an effect which can be utilised within an eating pattern to decrease overall intake and assist weight control or weight loss.

### 7.3 Outcomes and Disease Markers

The studies within this thesis related to health claims associated with satiety, overweight and obesity, with the PASSCLAIM criteria <sup>36</sup> used as a framework for measurement of outcomes and disease markers. The target functions/outcomes which are described include: reduces the risk of body weight gain, contributes to body weight reduction, decreases body fat and reduces abdominal fat. Markers which we seek to modify in relation to overweight are: helps to reduce energy/food intake, reduces appetite, increases satiety, increases metabolic rate/energy expenditure and increases lipid oxidation. Table 7.1 specifically outlines the outcome measures related to the studies performed in the thesis which were shown to be altered by  $\beta$ -glucan.

**Table 7-1 Target functions and outcome measures affected by  $\beta$ -glucan**

Target Functions	Meal Test Study	3 Month Trial
Contributes to body weight reduction	-	<i>weight loss*</i>
Decreases body fat	-	leptin*, insulin*
Reduces abdominal fat	-	<i>waist circumference*</i>
Helps reduce energy/food intake	<i>compare ad libitum food intake between groups<sup>#</sup></i>	<i>compare energy intakes between groups*</i>
Reduces appetite	<b>VAS</b> , ghrelin, <b>CCK</b> , <b>PYY</b>	VAS, ghrelin, CCK*, GLP-1*, PYY, PYY* <sub>3-36</sub>
Increases satiety	<b>VAS</b> , ghrelin, <b>CCK</b> , <b>PYY</b>	VAS, ghrelin, CCK*, GLP-1*, PYY, PYY* <sub>3-36</sub>

*Italics:* those measures which may be used as evidence of effect in relation to overweight and obesity. Other outcome markers/measures inform a mechanism by which the food component may have an effect.

**Bold:** those measures which were affected by  $\beta$ -glucan in the studies under consideration within this thesis.

<sup>#</sup> A trend indicated positive effects but they were not statistically significant.

\* these outcome measures improved but the effect was not different to that seen with an intervention of energy restriction alone.

## 7.4 $\beta$ -glucan in satiety and weight management

The research within this thesis adds to the evidence in support of a role of  $\beta$ -glucan as a stimulus for altered appetite hormones and adds to the body of mechanistic evidence related to fibres and weight control. In summary the key findings of this thesis are:

- A dose response exists between the amount of  $\beta$ -glucan consumed and plasma levels of insulin, CCK and PYY. This correlation is most pronounced with the appetite hormones.
- For PYY the effect is strongest at least 4 hours after ingestion of  $\beta$ -glucan.
- For effects of insulin, it is the first 2 hours which show dose responsiveness.
- A high dose of  $\beta$ -glucan (>5 g) tends to decrease intake at a subsequent meal.
- Consumption of cereals containing  $\beta$ -glucan increases subjective satiety at relatively low doses (approximately 2g).

- It is not possible to discern differences in weight loss over 3 months in an energy-restricted regimen between subjects consuming  $\beta$ -glucan up to 9g/day and subjects consuming a diet of moderate to high fibre.
- Extrusion is an acceptable form of processing of cereal products with  $\beta$ -glucan and cereals made in this way are well tolerated and of high palatability.

It is important to consider the results obtained in this thesis together with the past literature, reviewing how differences may exist but also as explanation for both positive and negative results. The results obtained in this thesis in part help to explain some negative results found in other studies. For example, in those studies examining satiety, if hormones, subjective satiety and energy intake are not measured over a minimum of 4 hours<sup>96, 106</sup>, then differences between a  $\beta$ -glucan meal and a control may not be distinguished. The studies within this thesis indicate hormones such as PYY, may have the greatest satiety effect due to changes in the GIT which occur many hours after a meal. Because  $\beta$ -glucan has been heavily investigated for its promotion of decreased glycaemia, many studies have been over short time frames. Logically, in longer term promotion of decreased energy intake and weight control, many foods could promote satiety over the two hours of a GI study, but helping individuals to stop consuming after this time point is most likely to be more important.

The fact that studies such as that by Juvonen and colleagues<sup>105</sup> found increased anorexigenic hormones (CCK, PYY, GLP-1) with low viscosity drinks over shorter time frames shows that viscosity is important. However, the fact that the high viscosity drink in the study promoted decreased energy intake over the day, indicates that high viscosity is most important in achieving decreased energy intake and hopefully weight loss or weight control. Therefore, studies which do not report viscosity, MW and solubility may not have used products which are likely to promote the formation of viscous boluses within the GIT. Studies on products that have no effect<sup>95, 103</sup> do not necessarily show that high viscosity  $\beta$ -glucan was ineffective if the physical characteristics were not measured. Just as sources of fibre vary in physiological effects<sup>220</sup>, processing, storage and food preparation techniques will further affect oat  $\beta$ -glucan. Studies using raw oats are likely to still have high MW and high viscosity. However, the limits of our detection methods for changes in markers and outcomes over the short time frames and with lower doses of  $\beta$ -glucan as in these studies<sup>100</sup> mean



positive outcomes may be lost. In addition, there is decreased solubility of raw oats decreasing its ability to form a viscous bolus in the GIT.

## 7.5 Future Directions

To develop a dossier related to satiety and weight control for  $\beta$ -glucan as a functional ingredient, the outcomes found in this thesis, combined with the literature identify the following key points and raise important requirements and questions for future research.

1. The mechanism of  $\beta$ -glucan's promotion of satiety most likely relates to stimulation of hormones such as CCK and PYY. The latter is in response to altered nutrient transit in the distal end of the GIT many hours after ingestion, so studies less than 4 hours are unlikely to show any effects. All future work should use 4 hours as the minimum time frame with collection of dietary intake data over the entire day.
2. Insulin and glycaemic effects are additional benefits and these can be measured over 2 hours. The reduction in insulin release may be modulated by increased release of GLP-1 and this may have positive effects on insulin resistance and other metabolic controls in the longer term, but this mechanism requires further research, measuring GLP-1 in further acute meal-test studies.
3.  $\beta$ -glucan decreases hunger over 4 hours and appetite hormones may mediate this effect, but this does not necessarily translate into reduction in food intake. However, trends revealed here together with the literature would indicate that at high doses (a minimum of 5g), energy intake is decreased and perhaps studied over an entire day (with as much as 10g  $\beta$ -glucan), positive results can be achieved.
4. The effects of  $\beta$ -glucan on weight control are subtle and unlikely to be measurable over short time frames such as in a 3-month trial. However, after 3 or more months of intervention, future studies should examine hormonal responses to meals (rather than fasting levels) which may indicate subtle changes in hormones such as PYY, CCK and GLP-1. These changes over greater time periods may assist in weight control.
5. When  $\beta$ -glucan is included in a weight-reducing regimen improved satiety and improved dietary compliance, with less withdrawals is sometimes observed. While there is insufficient evidence that  $\beta$ -glucan alone mediates weight loss,

changes in appetite hormones, subjective satiety and food intake are skewed in a positive direction in terms of longer term weight control. Further research over longer time frames is required.

There is evidence that  $\beta$ -glucan promotes satiety. There is insufficient evidence to support a health claim related to weight control at this point in time.

## REFERENCES

- 1 Australian Bureau of Statistics. National Health Survey 2007-2008: Summary of Results Cat. No. 4364.0, released 11/05/09. Canberra, 2009.
- 2 Burton-Freeman B. Dietary Fibre and Energy Regulation- Symposium: Dietary Composition and Obesity: do we need to look beyond dietary fat. *American Society for Nutritional Sciences*. 2000: 272S-75S.
- 3 Bazzano L, Song, Y., Bubes, V., Good, C., Manson, J. and Liu, S. Dietary intake of whole and refined grain breakfast cereals and weight gain in men. *Obesity Research*. 2005; **13**: 1952-60.
- 4 FDA. Nutrition Labeling of Food; Caloric content. *Federal Register*. 1987; **52**: 28590-691.
- 5 De Vries JHM. On defining dietary fibre. *Proceedings of the Nutrition Society*. 2003; **62**: 37-43.
- 6 Burkitt DP, Walker ARP, Painter NS. Effect of dietary fibre on stools and transit times, and its role in the causation of disease. *Lancet*. 1972; **ii**.
- 7 Trowell H. Crude fibre, dietary fibre and atherosclerosis. *Atherosclerosis*. 1972a; **16**: 138.
- 8 Trowell H. Ischemic heart disease and dietary fibre. *American Journal of Clinical Nutrition*. 1972b; **25**: 926-32.
- 9 Painter NS. Diverticular Disease of the Colon: A Deficiency Disease of Western Civilization. *London: Heinemann*. 1975.
- 10 Howarth NC, Saltzman E, Roberts SB. Dietary fiber and weight regulation. *Nutrition Reviews*. 2001; **59**: 129-39.
- 11 Lairon D, Arnault N, Bertrais S, *et al*. Dietary fiber intake and risk factors for cardiovascular disease in French adults. *American Journal of Clinical Nutrition*. 2005; **82**: 1185-94.
- 12 Brennan CS. Dietary fibre, glycaemic response and diabetes. *Molecular and Nutrition Food Research*. 2005; **49**: 560-70.
- 13 Levi F, Pasche C, LaVecchia C, Lucchini F, Franceschi S. Food groups and colorectal cancer risk. *British Journal of Cancer*. 1999; **79**: 1283-87.
- 14 Levi F, Pasche C, Lucchini F, Chatenoud L, Jacobs DR, La Vecchia C. Refined and whole grain cereals and the risks of oral, oesophageal and laryngeal cancer. *European Journal of Clinical Nutrition*. 2000; **54**: 487-89.
- 15 Gordon DT. Dietary fiber definitions at risk. *Cereal Foods World*. 2007; **52**: 112-23.

- 16 Englyst KN, Englyst HN. Carbohydrate bioavailability. *British Journal of Nutrition*. 2005; **94**: 1-11.
- 17 American Association of Cereal Chemists. The Definitions of Dietary Fibre. *Cereal Foods World*. 2001; **46**: 112-26.
- 18 Codex Alimentarius Commission. Report of the 30th session of the Codex Committee on nutrition and foods for special dietary uses, ALINORM 09/32/26 - appendix II. 2008; 46-.
- 19 European Parliament. COMMISSION DIRECTIVE 2008/100/EC of 28 October 2008 amending Council Directive 90/496/EEC on nutrition labelling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions. *The Official Journal of the European Union*. 2008; **L 285**: 9-12.
- 20 Food and Nutrition Board. Dietary Reference Intakes proposed definition of dietary fiber. *Institute of Medicine of the National Academies Washington, DC: The National Academies Press*. 2002: pp.7-1-7-69.
- 21 Slavin J. Impact of the proposed definition of dietary fiber on nutrient databases. *Journal of Food Composition and Analysis*. 2003; **16**: 287-91.
- 22 McCleary BV, DeVries J, Cohen JC, *et al*. Interlaboratory evaluation of an integrated procedure for the measurement of dietary fiber. *Cereal Foods World*. 2008; **53**: A35.
- 23 Codex Alimentarius. Guidelines for Use of Nutrition Claims: Draft Table of Contents for Nutrient Contents (Part B Containing Provisions on Dietary Fibre) (ALINORM 08/31/6, para. 41 and Appendix II). 2008.
- 24 FSANZ. A-277 Inulin and fructooligosaccharides as dietary fibre. Canberra: Food Standards Australia New Zealand, 2000.
- 25 FSANZ. A491 Resistant maltodextrin as dietary fibre. Canberra: Food Standards Australia New Zealand, 2004.
- 26 FSANZ. Nutrition, Health and Related Claims. A guide to the development of a food standard for Australia and New Zealand. Canberra: Food Standards Australia New Zealand, 2005.
- 27 FSANZ. Final Assessment Report Proposal 293, Nutrition, Health and Related Claims. Canberra: Food Standards Australia New Zealand, 2008; 1-141.
- 28 FSANZ. Proposal P293 Nutrition, Health and Related Claims: Consultation paper for first review 20 March 2009, [4-09]. 2009: 134.
- 29 FSANZ. Draft Assessment Report for Proposal 293: Nutrition, Health and Related Claims. Attachment 8, Substantiating Nutrition, Health and Related Claims on Foods. 2005.
- 30 Aggett PJ, Antoine J-M, Asp N-G, *et al*. PASSCLAIM Consensus on Criteria. *European Journal of Nutrition*. 2005; **44 Supplement 1**: 1/5-1/30.

- 31 Jones P, Silva P. Evidence for Health Claims on Food: How Much is Enough? *Journal of Nutrition*. 2008; **138**: 1189S-250S.
- 32 Yamada K, Sato-Minto N, Nagat J, Umegaki K. Health claim evidence requirements in Japan. *The Journal of Nutrition*. 2008; **138**: 1192S-98S.
- 33 Yang Y. Scientific substantiation of functional food health claims in China. *The Journal of Nutrition*. 2008; **138**: 1199S-205S.
- 34 Tapsell LC. Evidence for health claims: a perspective from the Australia-New Zealand Region. *The Journal of Nutrition*. 2008; **139**: 1206S-09S.
- 35 Food Standards Australia New Zealand. Draft Assessment Report for Proposal 293: Nutrition, Health and Related Claims. 2005a; **Attachment 8, Substantiating Nutrition, Health and Related Claims on Foods**.
- 36 Riccardi G, Aggett P, Brighenti F, *et al.* PASSCLAIM - Body weight regulation, insulin sensitivity and diabetes risk. *European Journal of Nutrition*. 2004; **43**: II/7-II/46.
- 37 European Parliament. Corrigendum to Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. *Official Journal of the European Journal*. 2007: 12/3-12/18.
- 38 Spiller RC. Pharmacology of dietary fibre. *Pharmacology and Therapeutics*. 1994; **62**: 407-27.
- 39 Miller WC, Niderpruem MG, Wallace JP, Lindeman AK. Dietary fat, sugar, and fiber predict body fat content. *Journal of the American Dietetic Association*. 1994; **94**: 612-15.
- 40 Lovejoy J, DiGirolamo M. Habitual dietary intake and insulin sensitivity in lean and obese adults. *American Journal of Clinical Nutrition*. 1992; **55**: 1174-79.
- 41 Alfieri MAH, Pomerleau J, Grace DM, Anderson L. Fiber intake of normal weight, moderately obese and severely obese subjects. *Obesity Research*. 1995; **3**: 541-47.
- 42 Davis JN, Hodges VA, Gillham MB. Normal-weight adults consume more fiber and fruit than their age- and height-matched overweight/obese counterparts. *Journal of the American Dietetic Association*. 2006; **106**: 833-40.
- 43 Kromhout D, Bloemberg B, Seidell JC, Nissinen A, Menotti A. Physical activity and dietary fiber determine population body fat levels: the Seven Countries Study. *International Journal of Obesity*. 2001; **25**: 301-06.
- 44 Rose N, Hosig K, Davy B, Serrano E, Davis L. Whole-Grain intake is associated with body mass index in college students. *Journal of Nutrition Education and Behavior*. 2007; **39**: 90-94.
- 45 Koh-Banerjee P, Franz M, Sampson L, *et al.* Changes in whole-grain, bran and cereal fiber consumption in relation to 8-y weight gain among men. *American Journal of Clinical Nutrition*. 2004; **80**: 1237-45.

- 46 Liu S, Willet W, Manson JW, Hu F, Rosner B, Colditz G. Relation between changes in intakes of dietary fiber and grain products and changes in weight and development of obesity among middle-aged women. *American Journal of Clinical Nutrition*. 2003; **78**: 920-27.
- 47 Ludwig DS, Pereira MA, Kroenke CH, *et al*. Dietary fiber, weight gain, and cardiovascular disease risk factors in young adults. *Journal of the American Medical Association*. 1999; **282**: 1539-46.
- 48 Montonen J, Knekt P, Jurvinen R, Aromaa A, Reunanen A. Whole-grain and fiber intake and the incidence of type 2 diabetes. *The American Journal of Clinical Nutrition*. 2003; **77**: 622-29.
- 49 Heaton KW. Dietary fibre - after 21 years of study the verdict remains one of fruition and frustration. *British Medical Journal*. 1990; **300**: 1479-80.
- 50 Leach JD. Evolutionary perspective on dietary intake of fibre and colorectal cancer. *European Journal of Clinical Nutrition*. 2007; **61**: 140-42.
- 51 Jones JM. Dietary fibre or whole grains or both? In: Salovaara H, Gates, F. and Tenkanen, M., editor. *Dietary Fibre Components and Functions*. Wageningen: Wageningen Academic Publishers, 2007; 13-30.
- 52 Valle-Jones JC. An open study of oat bran meal biscuits ('LeFibre') in the treatment of constipation in the elderly. *Current Medical Research and Opinion*. 1985; **9**: 716-20.
- 53 Lia A, Sundberg B, Aman P, Sandberg AS, Gallmans G, Andersson H. Substrates available for colonic fermentation from oat, barley and wheat bread diets. A study in ileostomy subjects. *British Journal of Nutrition*. 1996; **76**: 797-808.
- 54 Holt SH, Miller JC, Petocz P, Farmakalidis E. A satiety index of common foods. *European Journal of Clinical Nutrition*. 1995; **49**: 675-90.
- 55 Berti CR, P., Brusamolino A, Porrini M. Effect on appetite control of minor cereal and pseudocereal products. *British Journal of Nutrition*. 2005; **94**: 850-58.
- 56 Krotkiewski M. Use of fibres in different weight reduction programs. In: Bjoerntorp P, and Kritchevsky, G.V., editor. *Dietary Fibre and Obesity*. New York: Alan R. Liss, Inc., NY, 1985; 85-109.
- 57 American Dietetic Association. Position of the American Dietetic Association: Health implications of dietary fiber. *Journal of the American Dietetic Association*. 2002; **102**: 993-1000.
- 58 Stevens J, Ahn, K., Juhaeri, Houston, D., Steffan, L., Cooper, D. Dietary Fibre Intake and Glycaemic Index and incidence of diabetes in African-American and White Adults. *Diabetes Care*. 2003; **25**: 1715-21.
- 59 Knowler WC, Barrett-Connor E, Fowler S, *et al*. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *The New England Journal of Medicine*. 2002; **346**: 393-403.

- 60 Tuomilehto J, Lindstrom J, Eriksson J, *et al.* Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *The New England Journal of Medicine*. 2001; **344**: 1343-50.
- 61 Astrup A. Healthy lifestyles in Europe: prevention of obesity and type 2 diabetes by diet and physical activity. *Public Health Nutrition*. 2001; **4**: 499-515.
- 62 Ludwig DS. Dietary glycemic index and obesity. *Journal of Nutrition*. 2000; **130**: 280S-83S.
- 63 Jenkins DJA, Wolever TMS, Leeds AR, *et al.* Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity. *British Medical Journal*. 1978; **1**: 1392-94.
- 64 Battilana P, Ornstein K, Minehira K, *et al.* Mechanisms of action of beta-glucan in postprandial glucose metabolism in healthy men. *European Journal of Clinical Nutrition*. 2001; **55**: 327-33.
- 65 Björklund M, van Rees A, Mensink RP, Onning G. Changes in serum lipids and postprandial glucose and insulin concentrations after consumption of beverages with beta-glucans from oats or barley: a randomised dose controlled trial. *European Journal of Clinical Nutrition*. 2005; **59**: 1272-81.
- 66 Tappy L, Gugolz E, Wursch P. Effects of breakfast cereals containing various amounts of beta-glucan fibers on plasma glucose and insulin responses in NIDDM subjects. *Diabetes Care*. 1996; **19**: 831-34.
- 67 Ribordy V, Gremaud G, Meirim I, Piguet-Welsch C, Decombaz J. Effet de l'ingestion avant l'effort d'une barre cereale riche en son d'avoine sur le transit gastrointestinal et sur la glycémie (Effect of a pre-exercise cereal bar rich in oat bran on gastrointestinal transit and glycemia). *Schweizerische Zeitschrift für Sportmedizin und Sporttraumatologie*. 1997; **45**: 174-78.
- 68 Juntunen K, Niskanen L, Liukkonen K, Poutanen K, Holst J, Mykkanen H. Postprandial glucose, insulin, and incretin responses to grain products in healthy subjects. *American Journal of Clinical Nutrition*. 2002; **75**: 254-62.
- 69 Bornet FRJ, Jardy-Gennetier A-E, Jacquet N, Stowell J. Glycaemic response to foods: Impact on satiety and long-term weight regulation. *Appetite*. 2007; **49**: 535-53.
- 70 Jenkins DJA, Axelsen M, Kendall CWC, Augustin LSA, Vuksan V, Smith U. Dietary fibre, lente carbohydrates and the insulin-resistant diseases. *British Journal of Nutrition*. 2000; **83**: S157-S63.
- 71 Malkki Y, Virtanen E. Gastrointestinal effects of oat bran and oat gum - a review. *Lebensm-Wiss u-Technol*. 2001; **34**: 337-47.
- 72 Cervantes-Martinez C, Frey K, White P, Wesenberg D, Holland J. Correlated responses to selection for greater beta-glucan content in two oat populations. *Crop Science*. 2002; **42**: 730-38.

- 73 Wu YV, Doehlert DC. Enrichment of beta-glucan in oat bran by fine grinding and air classification. *Lebensm-Wiss u-Technol*. 2002; **35**: 30-33.
- 74 Nicolosi R, Bell S, Bistrian B, Greenberg I, Armour Forse R, Blackburn G. Plasma lipid changes after supplementation with beta-glucan fibre from yeast. *American Journal of Clinical Nutrition*. 1999; **70**: 208-12.
- 75 Amundsen AL, Haugum B, Andersson H. Changes in serum cholesterol and sterol metabolites after intake of products enriched with an oat bran concentrate within a controlled diet. *Scandinavian Journal of Nutrition*. 2003; **47**: 68-74.
- 76 Davidson M, Dugan L, Burns J, Bova J, Story K, Drennan K. The hypocholesterolemic effects of beta-glucan in oatmeal and oat bran. A dose-controlled study. *The Journal of the American Medical Association*. 1991; **265**.
- 77 Braaten JT, Wood PJ, Scott FW, *et al*. Oat beta-glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. *European Journal of Clinical Nutrition*. 1994; **48**: 465-74.
- 78 Uusitupa M, Ruuskanen E, Mäkinen E, *et al*. A controlled study on the effect of beta-glucan rich oat bran on serum lipids in hypercholesterolemic subjects: relation to apolipoprotein E phenotype. *Journal of the American College of Nutrition*. 1992; **11**: 651-59.
- 79 Kerckhoffs D, Brouns F, Hornstra G, Mensink R. Effects on the human serum lipoprotein profile of beta-glucan, soy protein and isoflavones, plant sterols and stanols, garlic and tocotrienols. *Journal of Nutrition*. 2002; **132**: 2494-505.
- 80 Ripsin CM, Keenan JM, Jacobes DR, *et al*. Oat products and lipid lowering. A meta-analysis. *Journal of the American Medical Association*. 1992; **267**: 3317-25.
- 81 Theuvsen E, Mensink RP. Water-soluble dietary fibers and cardiovascular disease. *Physiology and Behavior*. 2008; **94**: 285-92.
- 82 Jones PJH. Dietary agents that target gastrointestinal and hepatic handling of bile acids and cholesterol. *Journal of Clinical Lipidology*. 2008; **2**: S4-S10.
- 83 Ellegård L, Andersson H. Oat bran rapidly increases bile acid excretion and bile acid synthesis: and ileostomy study. *European Journal of Clinical Nutrition*. 2007; **61**: 938-45.
- 84 Behall KM, Scholfield DJ, Hallfrisch J. Effect of beta-glucan level in oat fiber extracts on blood lipids in men and women. *Journal of the American College of Nutrition*. 1997; **16**: 46-51.
- 85 Braaten JT, Wood PJ, Scott FW, *et al*. High beta-glucan oat bran and oat gum reduce postprandial blood glucose and insulin in subjects with and without type 2 diabetes. *Diabetes Medicine*. 1994; **11**: 312-18.
- 86 Behall KM, Scholfield DJ, Hallfrisch J, Liljeberg-Elmstahl HGM. Consumption of both resistant starch and beta-glucan improves postprandial plasma glucose and insulin in women. *Diabetes Care*. 2006; **29**: 976-81.



- 87 Rytter E, Erlanson-Albertsson C, Lindahl L, *et al.* Changes in plasma insulin, enterostatin, and lipoprotein levels during an energy-restricted dietary regimen including a new oat-based liquid food. *Annals of Nutrition and Metabolism*. 1996; **40**: 212-20.
- 88 Jenkins AL, Jenkins DJ, Zdravkovic U, Wursch P, Vuksan V. Depression of the glycemic index by high levels of beta-glucan fiber in two functional foods tested in type 2 diabetes. *European Journal of Clinical Nutrition*. 2002; **56**: 622-8.
- 89 Hallfrisch J, Schofield DJ, Behall KM. Diets containing soluble oat extracts improve glucose and insulin responses of moderately hypercholesterolemic men and women. *American Journal of Clinical Nutrition*. 1995; **61**: 379-84.
- 90 Wood PJ. Cereal beta-glucans in diet and health. *Journal of Cereal Science*. 2007; **46**: 230-38.
- 91 Wursch P, Pi-Sunyer FX. The role of viscous soluble fiber in the metabolic control of diabetes. A review with special emphasis on cereals rich in beta-glucan. *Diabetes Care*. 1997; **20**: 1774-80.
- 92 Makelainen H, Anttila HS, J., Hietanen R-M, *et al.* The effect of beta-glucan on the glycemic and insulin index. *European Journal of Clinical Nutrition*. 2007; **61**: 779-85.
- 93 Flint A, Moller BK, Raben A, *et al.* The use of glycaemic index tables to predict glycaemic index of composite breakfast meals. *British Journal of Nutrition*. 2004; **91**: 979-89.
- 94 Kabir M, Oppert J-M, Vidal H, *et al.* Four-Week Low-Glycemic Index Breakfast With a Modest Amount of Soluble Fibers in Type 2 Diabetic Men. *Metabolism*. 2002; **51**: 819-26.
- 95 Howarth NC, Saltzman E, McCrory MA, *et al.* Fermentable and nonfermentable fiber supplements did not alter hunger, satiety or body weight in a pilot study of men and women consuming self-selected diets. *The Journal of Nutrition*. 2003; **133**: 3141-44.
- 96 Kim H, Behall KM, Vinyard B, Conway J. Short-term satiety and glycemic response after consumption of whole grains with various amounts of beta-glucan. *Cereal Foods World*. 2006; **51**: 29-33.
- 97 Chow J, Choe YS, Noss MJ, *et al.* Effect of a viscous fiber-containing nutrition bar on satiety of patients with type 2 diabetes. *Diabetes Research and Clinical Practice*. 2007; **76**: 335-40.
- 98 Davidson TL, Walls EK, Ferguson A, Mak J, Swithers SE. Food viscosity as a determinant of caloric compensation and utilization. *Appetite*. 2006; **46**: 348.
- 99 Keogh JB, Lau CWH, Noakes M, Bowen J, Clifton PM. Effects of meals with high soluble fibre, high amylose barley variant on glucose, insulin, satiety and thermic effect of food in healthy lean women. *European Journal of Clinical Nutrition*. 2007; **61**: 597-604.

- 100 Saltzman E, Moriguti J, Krupa Das S, *et al.* Effects of a cereal rich in soluble fiber on body composition and dietary compliance during consumption of a hypocaloric diet. *Journal of the American College of Nutrition*. 2001; **20**: 50-57.
- 101 Greenway F, O'Neil CE, Stewart L, Rood J, Keenan M, Martin R. Fourteen weeks of treatment with Viscofiber increased fasting levels of glucagon-like peptide-1 and peptide-YY. *Journal of Medicinal Food*. 2007; **10**: 720-24.
- 102 Reyna NY, Cano C, Bermudez VJ, *et al.* Sweetener and beta-glucans improve metabolic and anthropometrics variables in well controlled type 2 diabetic patients. *American Journal of Therapeutics*. 2003; **10**: 438-43.
- 103 Pick ME, Hawrysh ZJ, Gee MI, Toth E, Garg ML, Hardin RT. Oat bran concentrate bread products improve long-term control of diabetes: a pilot study. *Journal of the American Dietetic Association*. 1996; **96**: 1254-61.
- 104 Peters HPF, Boers HM, Haddeman E, Melnikov AM, Qvyjt F. No effect of added b-glucan or of fructooligosaccharide on appetite or energy intake. *American Journal of Clinical Nutrition*. 2009; **89**: 58-63.
- 105 Juvonen KR, Purhonen A-K, Salmenkallio-Marttila M, *et al.* Viscosity of oat bran-enriched beverages influences gastrointestinal hormonal responses in healthy humans. *The Journal of Nutrition*. 2009; **139**: 1-6.
- 106 Hlebowicz J, Darwiche G, Bjorgell O, Almer L-O. Effect of muesli with 4g oat beta-glucan on postprandial blood glucose, gastric emptying and satiety in healthy subjects: a randomized crossover trial. *Journal of the American College of Nutrition*. 2008; **27**: 4.
- 107 Anttila H, Sontag-Strohm T, Salovaara H. Viscosity of beta-glucan in oat products. *Journal of Agriculture and Food Science*. 2004; **13**: 80-87.
- 108 Wood PJ, Braaten JT, Scott FW, Riedel KD, Wolynetz MS, Collins MW. Effect of dose and modification of viscous properties of oat gum on plasma and insulin following an oral glucose load. *British Journal of Nutrition*. 1994; **72**: 731-43.
- 109 Chen J, He J, Wildman RP, Reynolds K, Streiffer RH, Whelton PK. A randomized controlled trial of dietary fiber intake on serum lipids. *European Journal of Clinical Nutrition*. 2006; **60**: 62-68.
- 110 Kerckhoffs D, Hornstra, G., Mensink, R. Cholesterol-lowering effect of beta-glucan from oat bran in mildly hypercholesterolemic subjects may decrease when beta-glucan is incorporated into bread and cookies. *American Journal of Clinical Nutrition*. 2003; **78**: 221-27.
- 111 Lan-Pidhainy X, Brummer Y, Tosh SM, Wolever TM, Wood PJ. Reducing beta-glucan solubility in oat bran muffins by freeze-thaw treatment attenuates its hypoglycemic effect. *Cereal Chemistry*. 2007; **84**: 512-17.
- 112 Wood PJ, Beer MU, Butler G. Evaluation of role of concentration and molecular weight of oat beta-glucan in determining effect of viscosity on plasma glucose and insulin following an oral glucose load. *British Journal of Nutrition*. 2000; **84**: 19-23.

- 113 Lazaridou A, Biliaderis CG. Molecular aspects of cereal beta-glucan functionality: physical properties, technological applications and physiological effects. *Jouranal of Cereal Science*. 2007; **46**: 101-18.
- 114 Tosh SM, Brummer Y, Wolever TMS, Wood PJ. Glycemic response to oat bran muffins treated to vary molecular weight of beta-glucan. *Cereal Chemistry*. 2008; **85**: 211-17.
- 115 Chandra R, Liddle SA. Cholecystokinin. *Current Opinion in Endocrinology, Diabetes and Obesity*. 2007; **14**: 63-67.
- 116 de Graaf C, Blom W, Smeets P, Stafleu A, Hendriks H. Biomarkers of satiation and satiety. *American Journal of Clinical Nutrition*. 2004; **79**: 946-61.
- 117 Havel PJ. Peripheral signals conveying metabolic information to the brain: short-term and long-term regulation of food intake and energy homeostasis. *Experimental Biology and Medicine*. 2001; **226**: 963-77.
- 118 Burton-Freeman B. Sex and cognitive dietary restraint influence cholecystokinin release and satiety in response to preloads varying in fatty acid composition and content. *Journal of Nutrition*. 2005; **135**: 1407-11.
- 119 Burton-Freeman B, Davis PA, Schneeman B. Plasma cholecystokinin is associated with subjective measures of satiety in women. *American Journal of Clinical Nutrition*. 2002; **76**: 659-67.
- 120 Bourdon I, Yokoyama W, Davis P, *et al*. Postprandial lipid, glucose, insulin, and cholecystokinin responses in men fed barley pasta enriched with beta-glucan. *American Journal of Clinical Nutrition*. 1999; **69**: 55-63.
- 121 Geleva D, Thomas W, Gannon MC, Keenan JM. A solubilized cellulose fiber decreases peak postprandial cholecystokinin concentrations after a liquid mixed meal in hypercholesterolemic men and women. *The Journal of Nutrition*. 2003; **133**: 2194-203.
- 122 Pfluger PT, Kampe J, Castaneda TR, *et al*. Effect of human body weight changes on circulating levels of peptide YY and peptide YY3-36. *The Journal of Clinical Endocrinology & Metabolism*. 2007; **92**: 583-88.
- 123 Batterham RL, Cowley, M.A., Small, C.J., Herzog, H., Cohen, M.A. and Deakin, C.L. Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature*. 2002; **418**: 650-54.
- 124 McGowan BMC, Bloom SR. Peptide YY and appetite control. *Current Opinion in Pharmacology*. 2004; **4**: 583-88.
- 125 Howarth NC, Saltzman E, Roberts S. Dietary fibre and weight regulation. *Nutrition Reviews*. 2001; **59**: 129-39.
- 126 Batterham RL, Cohen MA, Ellis SM, Le Roux CW, *et al*.. Inhibition of food intake in obese subjects by peptide YY3-36 *The New England Journal of Medicine*. 2003; **349**: 941-48.

- 127 Roth CL, Enriori PJ, Harz K, Woelfle J, Cowley MA, Reinehr T. Peptide YY is a regulator of energy homeostasis in obese children before and after weight loss. *The Journal of Clinical Endocrinology & Metabolism*. 2005; **90**: 6386-91.
- 128 Le Roux CW, Batterham RL, Aylwin SJB, *et al*. Attenuated peptide YY release in obese subjects is associated with reduced satiety. *Endocrinology*. 2005; **147**: 3-8.
- 129 Sloth B, Davidsen L, Holst JJ, Flint A, Astrup A. Effect of subcutaneous injections of PYY1-36 and PYY3-36 on appetite, ad libitum energy intake, and plasma free fatty acid concentration in obese males. *American Journal of Physiology, Endocrinology and Metabolism*. 2007; **293**: E604-09.
- 130 Chan JL, Stoyneva V, Kelesidis T, Raciti P, Mantzoros CS. Peptide YY levels are decreased by fasting and elevated following caloric intake, but are not regulated by leptin. *Diabetologia*. 2006; **49**: 169-73.
- 131 Murphy K, Bloom SR. Gut hormones and the regulation of energy homeostasis. *Nature*. 2006; **444**: 854-58.
- 132 Gutzwiller J, Goke B, Drewe J, *et al*. Glucagon-like peptide-1: a potent regulator of food intake in humans. *Gut*. 1999; **44**: 81-86.
- 133 Adam T, Westerterp-Plantenga, M. Glucagon-like peptide-1 release and satiety after a nutrient challenge in normal-weight and obese subjects. *British Journal of Nutrition*. 2005; **93**: 845-51.
- 134 Holst JJ, Deacon CF, Vilsboll T, Krarup T, Madsbad S. Glucagon-like peptide-1, glucose homeostasis and diabetes. *Trends in Molecular Medicine*. 2008; **14**: 161-68.
- 135 DeFronzo RA, Ratner RE, Han J, Kim DD. Effects of Exenatide (Exendin-4) on Glycemic Control and Weight Over 30 Weeks in Metformin-Treated Patients With Type 2 Diabetes. *Diabetes Care*. 2005; **28**: 1092-100.
- 136 Otto B, Spranger J, Benoit S, Clegg D, Tschop M. The many faces of ghrelin: new perspectives for nutrition research? *British Journal of Nutrition*. 2005; **93**: 765-71.
- 137 Hosoda H, Kojima M, Kangawa K. Ghrelin and the regulation of food intake and energy balance. *Molecular Interventions*. 2002; **2**: 494-503.
- 138 Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weighle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes*. 2001; **50**: 1714-19.
- 139 Barazzoni R, Zanetti M, Stebel M, Biolo G, Cattin L, Guamieri G. Hyperleptinemia prevents increased plasma ghrelin concentration during short-term moderate caloric restriction in rats. *Gastroenterology*. 2003; **124**: 1188-92.
- 140 Cummings DE, Foster KE. Ghrelin-leptin tango in body-weight regulation. *Gastroenterology*. 2003; **124**: 1532-35.
- 141 Cummings DE. Ghrelin and the short- and long-term regulation of appetite and body weight. *Physiology and Behavior*. 2006; **89**: 71-84.

- 142 Wu JT, Kral JG. Ghrelin - integrative neuroendocrine peptide in health and disease. *Annals of Surgery*. 2004; **249**: 464-74.
- 143 Monteleone P, Bencivenga R, Longobardi N, Serritella C, Maj M. Differential responses of circulating ghrelin to high fat or high carbohydrate meal in healthy women. *Journal of Clinical Endocrinology & Metabolism*. 2003; **88**: 5510-14.
- 144 Weickert MO, Spranger J, Holst JJ, *et al*. Wheat-fibre-induced changes of postprandial peptide YY and ghrelin responses are not associated with acute alterations of satiety *British Journal of Nutrition*. 2006; **96**: 795-98.
- 145 Tannous Dit El Khoury D, Obeid O, Azar S, Hwalla N. Variations in postprandial ghrelin status following ingestion of high carbohydrate, high fat and high protein meals in males. *Annals of Nutrition and Metabolism*. 2006; **50**: 260-69.
- 146 Feinle-Bisset C, Patterson M, Ghatti MA, Bloom SR, Horowitz M. Fat digestion is required for the suppression of ghrelin and stimulation of peptide YY and pancreatic polypeptide secretion by intraduodenal lipid. *American Journal of Physiology, Endocrinology and Metabolism*. 2005; **289**: E948-53.
- 147 Havel PJ, Townsend R, Chaump L, Teff K. High-fat meals reduce 24-h circulating leptin concentrations in women. *Diabetes*. 1999; **48**: 334-41.
- 148 Koh-Banerjee P, Rimm EB. Whole grain consumption and weight gain a review of the epidemiological evidence, potential mechanisms and opportunities for future research. *Proceedings of the Nutrition Society*. 2003; **62**: 25-29.
- 149 Morton GJ. Hypothalamic leptin regulation of energy homeostasis and glucose metabolism. *Journal of Physiology*. 2007; **583**: 437-43.
- 150 Flint A, Gregersen T, Gluud LL, *et al*. Associations between postprandial insulin and blood glucose responses, appetite sensations and energy intake in normal weight and overweight individuals: a meta-analysis of test meal studies. *British Journal of Nutrition*. 2007; **98**: 17-25.
- 151 Weigle DS, Duell PB, Connor WE, Steiner RA, Soules MR, Kuijper JL. Effect of Fasting, Refeeding, and Dietary Fat Restriction on Plasma Leptin Levels. *Journal of Clinical Endocrinology and Metabolism*. 1997; **82**: 561-65.
- 152 Chin-Chance C, Polonsky KS, Schoeller DA. Twenty-four-hour leptin levels respond to cumulative short-term energy imbalance and predict subsequent intake. *The Journal of Clinical Endocrinology & Metabolism*. 2000; **85**: 2685-91.
- 153 Friedman JM. The function of leptin in nutrition, weight, and physiology. *Nutrition Reviews: Nineteenth Marabou Symposium*. 2002; **2** **60**: S68-84, 85-7.
- 154 Mattes RD, Hollis J, Hayes D, Stunkard AJ. Appetite: measurement and manipulation misgivings. *Journal of the American Dietetic Association*. 2005; **105**: S87-S97.

- 155 Doucet E, St-Pierre S, Almeras N, Tremblay A. Relation between appetite ratings before and after a standard meal and estimates of daily energy intake in obese and reduced obese individuals. *Appetite*. 2003; **40**: 137-43.
- 156 Parker BA, Sturm K, MacIntosh CG, Feinle C, Horowitz M, Chapman IM. Relation between food intake and visual analogue scale ratings of appetite and other sensations in healthy older and young subjects. *European Journal of Clinical Nutrition*. 2004; **58**: 212-18.
- 157 Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *International Journal of Obesity*. 2000; **24**: 38-48.
- 158 Stubbs RJ, Hughes DA, Johnstone AM, *et al*. The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. *British Journal of Nutrition*. 2000; **84**: 405-15.
- 159 Cardello AV, Schutz HG, Leshner LL, Merrill E. Development and testing of a labeled magnitude scale of perceived satiety. *Appetite*. 2005; **44**: 1-13.
- 160 Stratton RJ, Stubbs RJ, Hughes D, King N, Blundell JE, Elia M. Comparison of the traditional paper visual analogue scale questionnaire with an Apple Newton electronic appetite rating system (EARS) in free living subjects feeding ad libitum. *European Journal of Clinical Nutrition*. 1998; **52**: 737-41.
- 161 Greene SM, Delargy HJ, Joanes D, Blundell JE. A satiety quotient: a formulation to assess the satiating effect of food. *Appetite*. 1997; **29**: 291-304.
- 162 Simpson SJ, Raubenheimer D. Obesity: the protein leverage hypothesis. *Obesity Reviews*. 2005; **6**: 133-42.
- 163 Weinsier RL, Nagy TR, Hunter GR, Darnell BE, Hensrud DD, Weiss HL. Do adaptive changes in metabolic rate favor weight regain in weight-reduced individuals? An examination of the set-point theory. *Am J Clin Nutr*. 2000; **72**: 1088-94.
- 164 Macias AE. Experimental demonstration of human weight homeostasis: implications for understanding obesity. *British Journal of Nutrition*. 2004; **91**: 479-84.
- 165 Major GC, Doucet E, Trayhurn P, Astrup A, Tremblay A. Clinical significance of adaptive thermogenesis. *International Journal of Obesity*. 2007; **31**: 204-12.
- 166 Hirsch JA, Herman CP. Obesity, dieting and the expression of 'obese' characteristics. *Journal of Comparative Physiology and Psychology*. 1977; **2**: 374-80.
- 167 Gregersen NT, Flint A, Bitz C, Blundell JE, Raben A, Astrup A. Reproducibility and power of ad libitum energy intake assessed by repeated single meals. *American Journal of Clinical Nutrition*. 2008; **87**.
- 168 Goldberg GR, Black AE, Jebb SA, *et al*. Critical evaluation of energy intake data using fundamental principles of energy physiology: 1 Derivation of cut-off limits to identify underreporting. *European Journal of Clinical Nutrition*. 1991; **45**: 569-81.

- 169 Hendrickson S, Mattes R. Financial Incentive for Diet Recall Accuracy Does Not Affect Reported Energy Intake or Number of Underreporters in a Sample of Overweight Females. *Journal of the American Dietetic Association*. 2007; **107**: 118-21.
- 170 de Lauzon-Guillain B, Basdevant A, Romon M, Karlsson J, Borys JM, Charles MA. Is restrained eating a risk factor for weight gain in a general population?. *American Journal of Clinical Nutrition*. 2006; **83**: 132-38.
- 171 Stice E, Cooper JA, Schoeller DA, Tappe K, Lowe MR. Are dietary restraint scales valid measures of moderate- to long-term dietary restriction? Objective biological and behavioral data suggest not. *Psychological Assessment*. 2007; **19**: 449-58.
- 172 Stunkard AJ, Messick S. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *Journal of Psychosomatic Research*. 1985; **29**: 71-83.
- 173 Raben A, Agerholm-Larsen L, Flint A, Holst J, Astrup A. Meals with similar energy densities but rich in protein, fat, carbohydrate, or alcohol have different effects on energy expenditure and substrate metabolism but not on appetite and energy intake. *American Journal of Clinical Nutrition*. 2003; **77**: 91-100.
- 174 Sorensen LB, Moller P, Flint A, Martens M, Raben A. Effects of sensory perception of foods on appetite and food intake: a review of studies on humans. *International Journal of Obesity and Related Metabolic Disorders*. 2003; **27**: 1152-66.
- 175 Norton GN, Anderson AS, Hetherington MM. Volume and variety: relative effects on food intake. *Physiology and Behaviour*. 2006.
- 176 Gerstein DE, Woodward-Lopez G, Evans A, Kelsey K, Drewnowski A. Clarifying concepts about macronutrients' effects on satiation and satiety. *Journal of the American Dietetic Association*. 2004; **104**: 1151-53.
- 177 Beasley JM, Ange BA, Anderson CAM, *et al.* Associations between macronutrient intake and self-reported appetite and fasting levels of appetite hormones: results from the Optimal Macronutrient Intake Trial to Prevent Heart Disease. *American Journal of Epidemiology*. 2009; **169**: 893-900.
- 178 Bowen J, Noakes M, Clifton PM. Appetite hormones and energy intake in obese men after consumption of fructose, glucose and whey protein beverages. *International Journal of Obesity*. 2007; **31**: 1696-703.
- 179 NHMRC. How to use the evidence: assessment and application of scientific evidence. National Health and Medical Research Council, Commonwealth of Australia, Canberra, ACT, 2000.
- 180 Sibbald B, Roland M. Understanding controlled trials: why are randomised controlled trials important. *British Medical Journal*. 1998; **316**: 201.
- 181 Gunn E, Bryant D, Somerville L, Birmingham T, Oxman AD. Outcomes of patients who participate in randomised controlled trials compared to similar patients receiving similar interventions who do not participate. *Cochrane Database of Systematic Reviews*. 2009; **2**.

- 182 McCleary BV, Glennie-Holmes M. Enzymatic quantification of (1→3) (1→4) -  $\beta$ -D-glucan in barley and malt. *Journal of the Institute of Brewery*. 1985; **91**: 285-95.
- 183 Beer MU, Wood PJ, Weisz J, Fillion N. Effect of cooking and storage on the amount and molecular weight of (1→3) (1→4)- $\beta$ -D-glucan extracted from oat products by an in vitro digestion system. *Cereal Chemistry*. 1997; **74**: 705-09.
- 184 Jørgensen KG. Quantification of high molecular weight (1→3) (1→4) -  $\beta$ -D-glucan using calcofluor complex formation and flow injection analysis. Analytical principle and its standardization. *Carlsberg Research Communications*. 1988; **53**: 287-96.
- 185 Wood PJ, Weisz J, Mahn W. Molecular characterization of cereal  $\beta$ -glucans. II. Size-exclusion chromatography for comparison of molecular weight. *Cereal Chemistry*. 1991; **68**: 530-36.
- 186 Flint A, Møller BK, Raben A, *et al.* Glycemic and insulinemic responses as determinants of appetite in humans. *American Journal of Clinical Nutrition*. 2006; **84**: 1365-73.
- 187 Wolever TM. Effect of blood sampling schedule and method of calculating the area under the curve on validity and precision of glycaemic index values. *British Journal of Nutrition*. 2004; **91**: 295-300.
- 188 Wolever TM, Brand Miller J, Abernethy J, *et al.* Measuring the glycaemic index of foods - interlaboratory study. *American Journal of Clinical Nutrition*. 2008; **87**: 247S-57S.
- 189 Aman P, Rimsten L, Andersson R. Molecular Weight Distribution of beta-glucan in oat-based foods. *Cereal Chemistry*. 2004; **81**: 356-60.
- 190 Holm J, Koellreutter B, Wursch P. Influence of sterilization drying and oat bran enrichment of pasta on glucose and insulin responses in healthy subjects and on the rate and extent of *in vitro* starch digestion. *European Journal of Clinical Nutrition*. 1992; **46**: 629-40.
- 191 Wolever TM, Bolognesi C. Source and amount of carbohydrate affect postprandial glucose and insulin in normal subjects. *Journal of Nutrition*. 1996; **126**: 2798-806.
- 192 Karpe F, Tan GD. Adipose tissue function in the insulin-resistance syndrome. *Biochemical Society Transactions*. 2005; **33(Pt 5)**: 1045-48.
- 193 Björck I, Liljeberg H, Ostman EM. Low Glycemic-Index Foods. *British Journal of Nutrition*. 2000; **83**: S149-S55.
- 194 Granfeldt Y, Nyberg L, Björck I. Muesli with 4g oat [ $\beta$ ]-glucans lowers glucose and insulin responses after a bread meal in healthy subjects. *European Journal of Clinical Nutrition*. 2008; **62**: 600-07.



- 195 Burton-Freeman B, Davis PA, Schneeman B. Interaction of fat availability and sex on postprandial satiety and cholecystokinin after mixed-food meals. *American Journal of Clinical Nutrition*. 2004; **80**: 1207-14.
- 196 Huang X-F, Yu Y, Beck EJ, South T, Batterham MJ, Tapsell LC. Oat  $\beta$ -glucan increases satiety via activation of the PYY3-36 -Y2 -NPY pathway. *submitted to Journal of Cellular Biochemistry*.
- 197 Sainsbury A, Schwarzer C, Couzens M, *et al*. Important role of hypothalamic Y2 receptors in body weight regulation revealed in conditional knockout mice. *Proceedings of the National Academy of Sciences of the United States of America*. 2002; **99**: 8938-43.
- 198 Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Deakin CL. Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature*. 2002; **418**: 650-54.
- 199 Beck EJ, Tosh SM, Batterham MJ, Tapsell LC, Huang XF. Oat  $\beta$ -glucan increases postprandial cholecystokinin levels, decreases insulin response and extends subjective satiety in overweight subjects. *Molecular Nutrition and Food Research*, *accepted 23 February 2009*. 2009.
- 200 Huang X-F, Yu Y, Beck EJ, South T, Batterham MJ, Tapsell LC. Oat  $\beta$ -glucan increases satiety via activation of the PYY3-36 -Y2 -NPY pathway. *submitted paper*.
- 201 Andersson AAM, Armo E, Grangeon E, Fredriksson H, Andersson R, Aman P. Molecular weight and structure units of (1 $\rightarrow$ 3, 1 $\rightarrow$ 4)- $\beta$ -glucans in dough and bread made from hull-less barley milling fractions. *Journal of Cereal Science*. 2004; **40**: 195-204.
- 202 Solum TT, Rytting KR, Solum E, Larsen S. The influence of a high-fibre diet on body weight, serum lipids and blood pressure in slightly overweight persons. A randomized, double-blind, placebo-controlled investigation with diet and fibre tablets. *International Journal of Obesity*. 1987; **11 S1**: 67-71.
- 203 Martin GS, Tapsell LC, Denmeade S, Batterham MJ. Relative validity of a diet history interview in an intervention trial manipulating dietary fat in the management of Type II diabetes mellitus. *Preventive Medicine*. 2003; **36**: 420-28.
- 204 Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *American Journal of Clinical Nutrition*. 1982; **36**: 936-42.
- 205 Selwyn AP. Weight reduction and cardiovascular and metabolic disease prevention: clinical trial update. . *American Journal of Cardiology*. 2007; **100** 33-37.
- 206 St-Onge MP, Desmond R, Hunter G, Gower B. Baseline inflammatory markers do not modulate the lipid response to weight loss. *Metabolism: Clinical & Experimental*. 2008; **57**: 598-604.
- 207 de Luis DA, Sagrado MG, Conde R, Aller R, Izaola O. Changes of ghrelin and leptin in response to hypocaloric diet in obese patients. *Nutrition*. 2008; **24**: 162-6

- 208 Norris SL, Zhang X, Avenell A, Gregg E, Schmid CH, Lau J. Long-term non-pharmacological weight loss interventions for adults with prediabetes. *Cochrane Database of Systematic Reviews*. 2009; **2**.
- 209 Misra A, Vikram NK. Clinical and pathophysiological consequences of abdominal adiposity and abdominal adipose tissue depots. *Nutrition*. 2003; **19**: 457-66.
- 210 Despres JP, Lemieux I, Prud'homme D. Treatment of obesity: need to focus on high risk abdominally obese patients. *British Medical Journal*. 2001; **322**: 716-20.
- 211 Reinehr T, Roth CL, Schernthaner G-H, Kopp H-P, Kriwanek S, Schernthaner G. Peptide YY and glucagon-like peptide-1 in morbidly obese patients before and after surgically induced weight loss. *Obesity Surgery*. 2007; **17**: 1571-77.
- 212 Adam TC, Jocken J, Westerterp-Plantenga MS. Decreased glucagon-like peptide 1 release after weight loss in overweight/obese subjects. *Obesity Research*. 2005; **13**: 710-16.
- 213 Salas-Salvado J, Farre X, Luque X, *et al*. Effect of two doses of a mixture of soluble fibres on body weight and metabolic variables in overweight or obese patients: a randomised trial. *British Journal of Nutrition*. 2008; **99**: 1380-87.
- 214 Alhassan S, Kim S, Bersamin A, King AC, Gardner CD. Dietary adherence and weight loss success among overweight women: results from the A TO Z weight loss study. *International Journal of Obesity*. 2008; **32**: 985-91.
- 215 FSANZ. About FSANZ Canberra: FSANZ 2008. ([Also available from: <http://www.foodstandards.gov.au/aboutfsanz/index.cfm> accessed 4 December 2008).
- 216 FSANZ. A277 Inulin and fructooligosaccharides as dietary fibre. Canberra: Food Standards Australia New Zealand, 2001.
- 217 FSANZ. Proposal 293 – Nutrition, Health & Related Claims, Attachment 13 Summary of submissions received in response to the Preliminary Final Assessment Report., 2005; 57-65.
- 218 Williams P, Member of 1994 National Food Authority Working Party on Nutrient Claims, personal communication. 2008.
- 219 European Food Safety Authority. Scientific and technical guidance for the preparation and presentation of the application for authorisation of a health claim. . *The EFSA Journal*. 2007; **530**: 1-44.
- 220 Slavin J, Klosterbuer A, Willis HJ. How fiber affects weight regulation. *Food Technology*. 2008; **62**: 34-41.

## **Appendix 3-1**

**Appendix 3-1 Table 1 Extruder variables for Samples produced at low Shear and variable temperatures.**

Formulation	HBGO LowShear/ LowTemperature		HBGO LowShear/ Med. Temperature		HBGO LowShear/ High Temperature		HBGX LowShear/ Low Temperature		HBGX LowShear/ Med. Temperature		HBGX LowShear/ High Temperature	
Temperature °C (Temp)	Temp Set	Temp Actual	Temp Set	Temp Actual	Temp Set	Temp Actual	Temp Set	Temp Actual	Temp Set	Temp Actual	Temp Set	Temp Actual
Stage 2	45	45	45	45	45	45	45	45	45	45	45	45
Stage 3	45	45	45	45	45	45	45	45	45	45	45	45
Stage 4	45	45	45	45	45	45	45	45	45	45	45	45
Stage 5	45	45	45	50	55	54	45	43	55	49	55	51
Stage 6	60	61	100	101	100	85	60	55	100	99	100	100
Stage 7	80	82	110	111	110	99	80	73	110	111	110	110
Stage 8	100	100	120	123	130	129	100	98	120	120	130	131
Stage 9	110	109	130	134	150	150	110	109	130	131	150	152
Stage 10	120	121	140	142	170	170	120	120	140	140	170	172
Head Temp °C	162		175		185		170		173		181	
Dry feed flow rate kg/hr	19.9152		20.034		19.953		19.85		20.061		19.845	
Wet feed set point*	9.7		9.7		7.2		7.04		7.1		7.1	
Wet feed flow rate L/hr	2.7		2.78		2.279		2.26		2.28		2.28	
% Screw speed	40		40		40		40		40		40	
% Torque	33		34		32		30		28		25	
Cutter Speed*	1.9		1.9		1.9		1.9		1.9		1.9	
Die Pressure (X 100 PSI)	1020		900		880-900		950-1050		800-950		720-820	
Bulk Densityg/L	262.3		219.2		178.6		180.6		177.2		144.3	
SME# kW.hr/kg	0.153		0.153		0.167		0.156		0.146		0.13	

\* machine specific arbitrary unit # specific mechanical energy (SME) = screw speed X Torque X KW (machine specific – 29)/ Total Input (kg/hr – water plus dryfeed)

**Appendix 3-1 table 2 Extruder variables for samples produced at medium shear and variable temperatures.**

Formulation	HBGO Med. Shear/ Low Temperature		HBGO Med. Shear/ Med. Temperature		HBGO Med. Shear/ High Temperature		HBGX Med. Shear/ Low Temperature		HBGX Med. Shear/ Med. Temperature		HBGX Med. Shear/ High Temperature	
Temperature °C (Temp)	Temp Set	Temp Actual	Temp Set	Temp Actual	Temp Set	Temp Actual	Temp Set	Temp Actual	Temp Set	Temp Actual	Temp Set	Temp Actual
Stage 2	45	45	45	45	45	45	45	45	45	45	45	45
Stage 3	45	43	45	45	45	45	45	45	45	45	45	45
Stage 4	45	45	45	45	45	46	45	45	45	45	45	44
Stage 5	45	45	45	63	55	66	45	38	55	50	55	56
Stage 6	60	60	100	97	100	101	60	58	100	99	100	100
Stage 7	80	82	110	112	110	113	80	78	110	109	110	110
Stage 8	100	100	120	123	130	132	100	98	120	119	130	130
Stage 9	110	109	130	134	150	154	110	107	130	127	150	151
Stage 10	120	121	140	143	170	173	120	120	140	138	170	171
Head Temp °C	173		176		187		169		175		182	
Dry feed flow rate kg/hr	19.969		19.98		19.877		20.336		20.128		19.845	
Wet feed set point	8.5		8.5		8.5		7.1		7.1		7.1	
Wet feed flow rate L/hr	2.568		2.598		2.577		2.306		2.295		2.312	
% Screw speed	40		40		40		40		40		40	
% Torque	36		38		34		32		30		31	
Cutter Speed	1.9		1.9		1.9		1.9		1.9		1.9	
Die Pressure (X 100 PSI)	980-1060		810-830		680-700		900-1040		880-980		680-720	
Bulk Density g/L	219.5		198.5		178.8		170.4		161.8		129.6	
SME kW.hr/kg	0.185		0.195		0.161		0.166		0.156		0.161	

**Appendix 3-1 table 3 Extruder variables for samples produced at high shear and variable temperatures.**

Formulation	HBGO High Shear/ Low Temperature		HBGO High Shear/ Med. Temperature		HBGO High Shear/ High Temperature		HBGX High Shear/ Low Temperature		HBGX High Shear/ Med. Temperature		HBGX High Shear/ High Temperature	
Temperature °C (Temp)	Temp Set	Temp Actual	Temp Set	Temp Actual	Temp Set	Temp Actual	Temp Set	Temp Actual	Temp Set	Temp Actual	Temp Set	Temp Actual
Stage 2	45	45	45	45	45	45	45	45	45	45	45	47
Stage 3	45	45	45	47	45	45	45	45	45	45	45	47
Stage 4	45	45	45	60	45	50	45	441	45	47	45	54
Stage 5	45	45	45	67	55	66	45	38	55	57	55	66
Stage 6	60	61	100	100	100	102	60	59	100	99	100	101
Stage 7	80	82	110	112	110	113	80	80	110	110	110	113
Stage 8	100	99	120	123	130	132	100	97	120	119	130	131
Stage 9	110	109	130	133	150	153	110	107	130	128	150	152
Stage 10	120	121	140	143	170	172	120	116	140	137	170	169
Head Temp °C	168		177		184		162		170		178	
Dry feed flow rate kg/hr	19.790		20.342		20.034		20.984		19.764		19.856	
Wet feed set point	8.5		8.6		8.6		7.1		7.1		7.1	
Wet feed flow rate L/hr	2.567		2.557		2.539		2.355		2.367		2.369	
% Screw speed	40		40		40		40		40		40	
% Torque	34		33		33		30		28		24	
Cutter Speed	1.9		1.9		1.9		1.9		1.9		2.1	
Die Pressure (X 100 PSI)	1040-1080		960-1020		760-800		860-980		780-900		660-740	
Bulk Density g/L	239.6		213.5		196.8		210.4		185.1		165.5	
SME kW.hr/kg	0.175		0.170		0.170		0.156		0.145		0.124	

**Appendix 3-1 table 4 Extruder variables for additional samples produced at low Shear and variable temperatures. \*less uniform product**

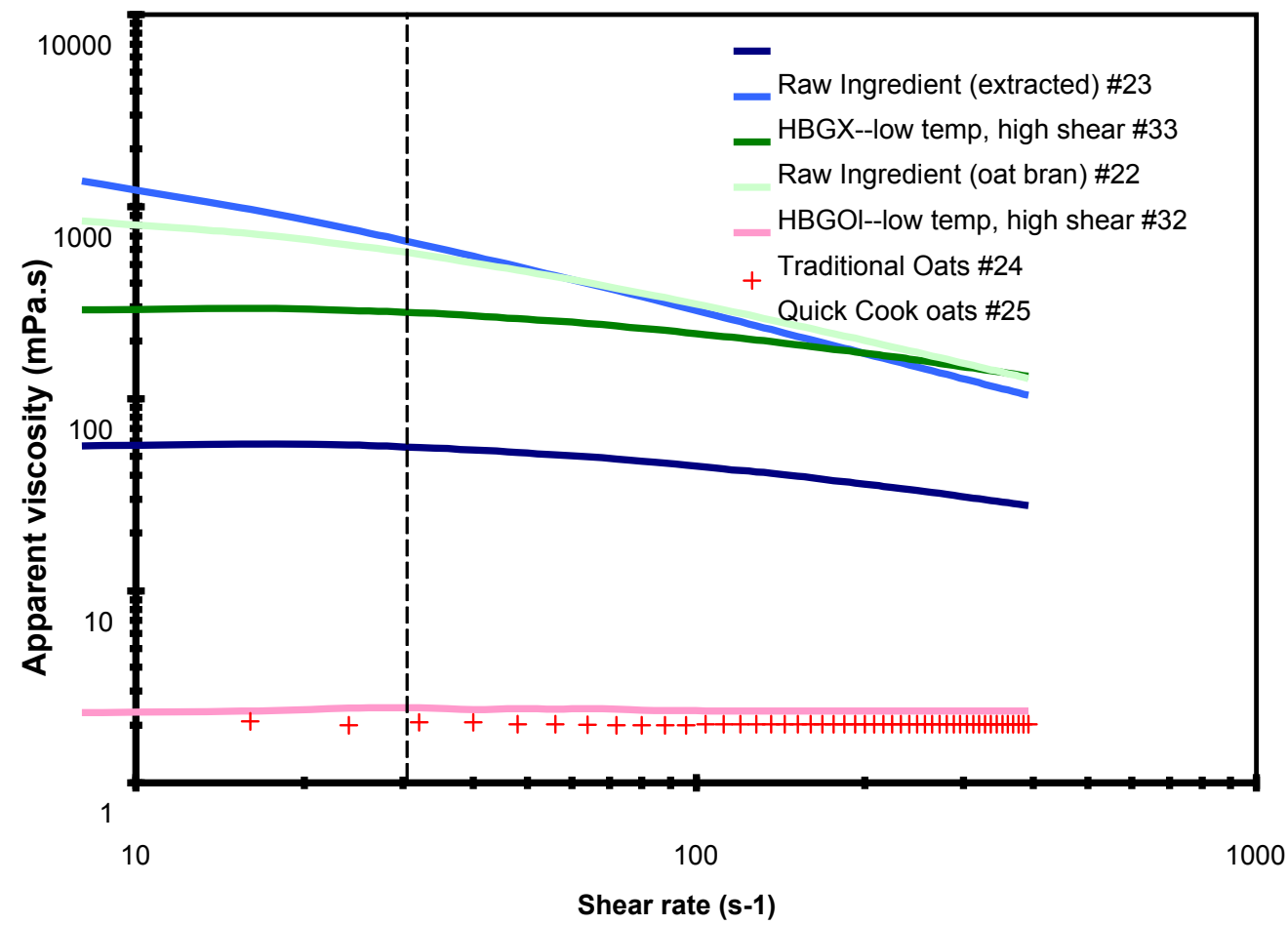
Formulation	Oat Bran (Raw) High Shear/ Low Temperature		Extracted $\beta$ -glucan (Raw) High Shear/ Med. Temperature		Extracted $\beta$ -glucan* Low Shear/Low Temp/ Low Screw Speed	
Temperature °C (Temp)	Temp Set	Temp Actual	Temp Set	Temp Actual	Temp Set	Temp Actual
Stage 2	45	45	45	45	45	45
Stage 3	45	45	45	45	45	45
Stage 4	45	45	45	44	45	45
Stage 5	45	45	45	43	45	44
Stage 6	60	64	60	59	60	59
Stage 7	80	82	80	79	80	77
Stage 8	100	101	100	102	100	99
Stage 9	110	110	110	114	110	110
Stage 10	120	121	120	124	120	119
Head Temp °C	183		197		156	
Dry feed flow rate kg/hr	19.996		19.996		19.962	
Wet feed set point	21		40.2		7.1	
Wet feed flow rate L/hr	5.12		8.678		2.249	
% Screw speed	60		60		25	
% Torque	30		34		37	
Cutter Speed	1.4		1.4		1.9	
Die Pressure (X 100 PSI)	700-740		820-920		1150-1390	
Bulk Density g/L	220.2		172.9		261.3	
SME kW.hr/kg	0.208		0.206		0.117	

**Appendix 3-2**

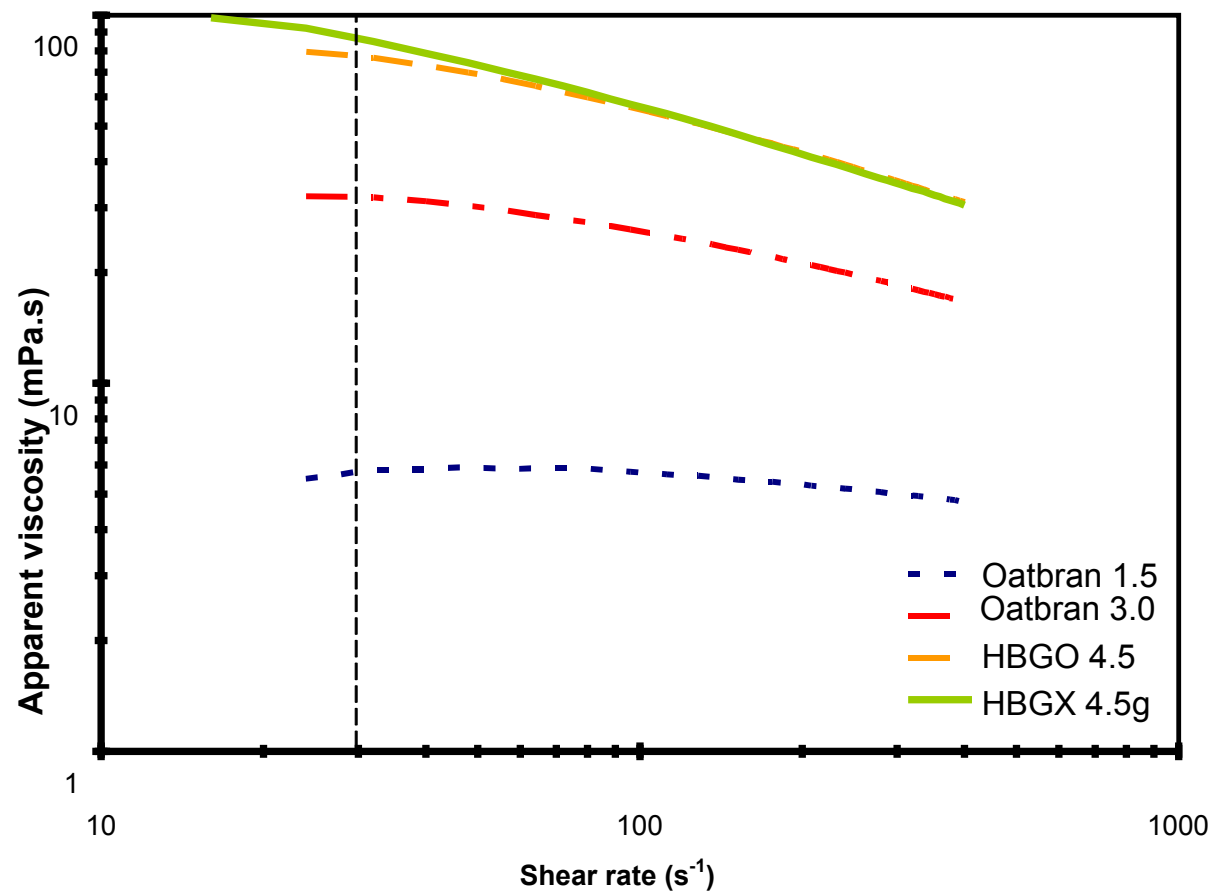
Graphical results for viscosity and solubility for study products produced by Dr Susan Tosh (Agriculture and Agri-Food Canada)



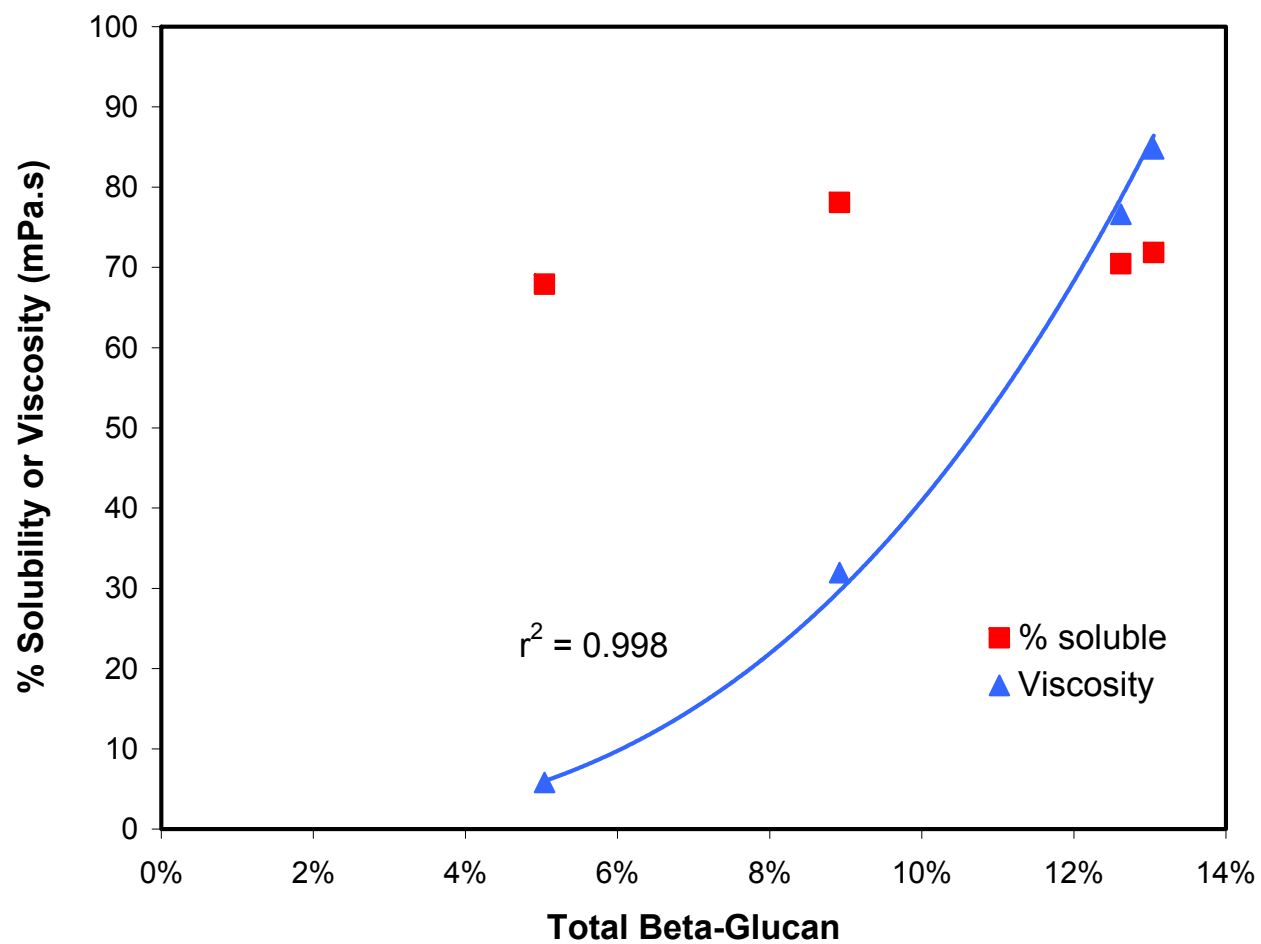
Appendix 3-2 Figure 1 -extract viscosity for ingredients (Shear thinning behaviour is demonstrated. Dotted line shows 30 s-1



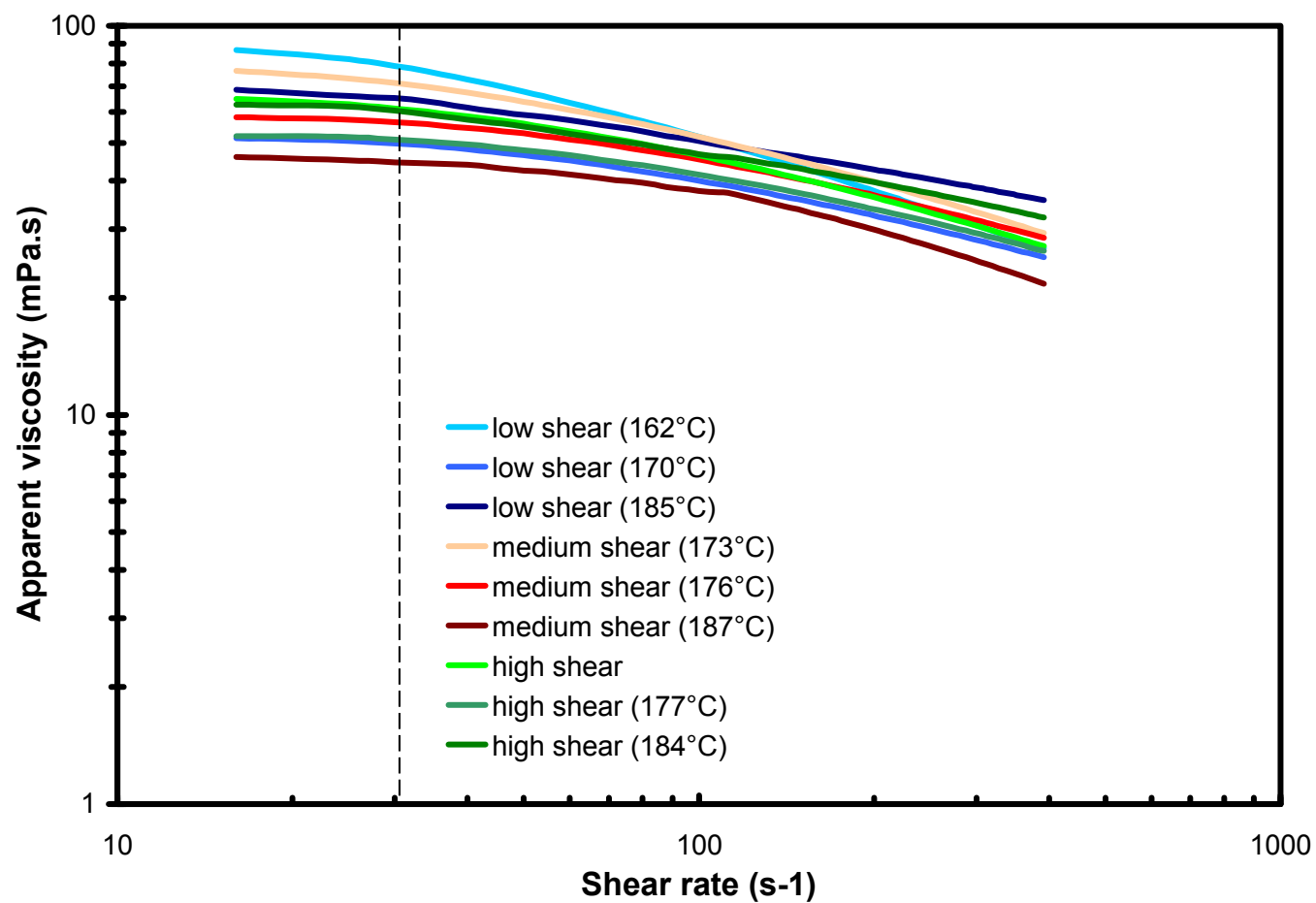
Appendix 3-2 Figure 2: Apparent viscosity of extracts from cereal containing different amounts of  $\beta$ -glucan (Shear thinning behaviour is demonstrated. Dotted line shows 30 s<sup>-1</sup>)



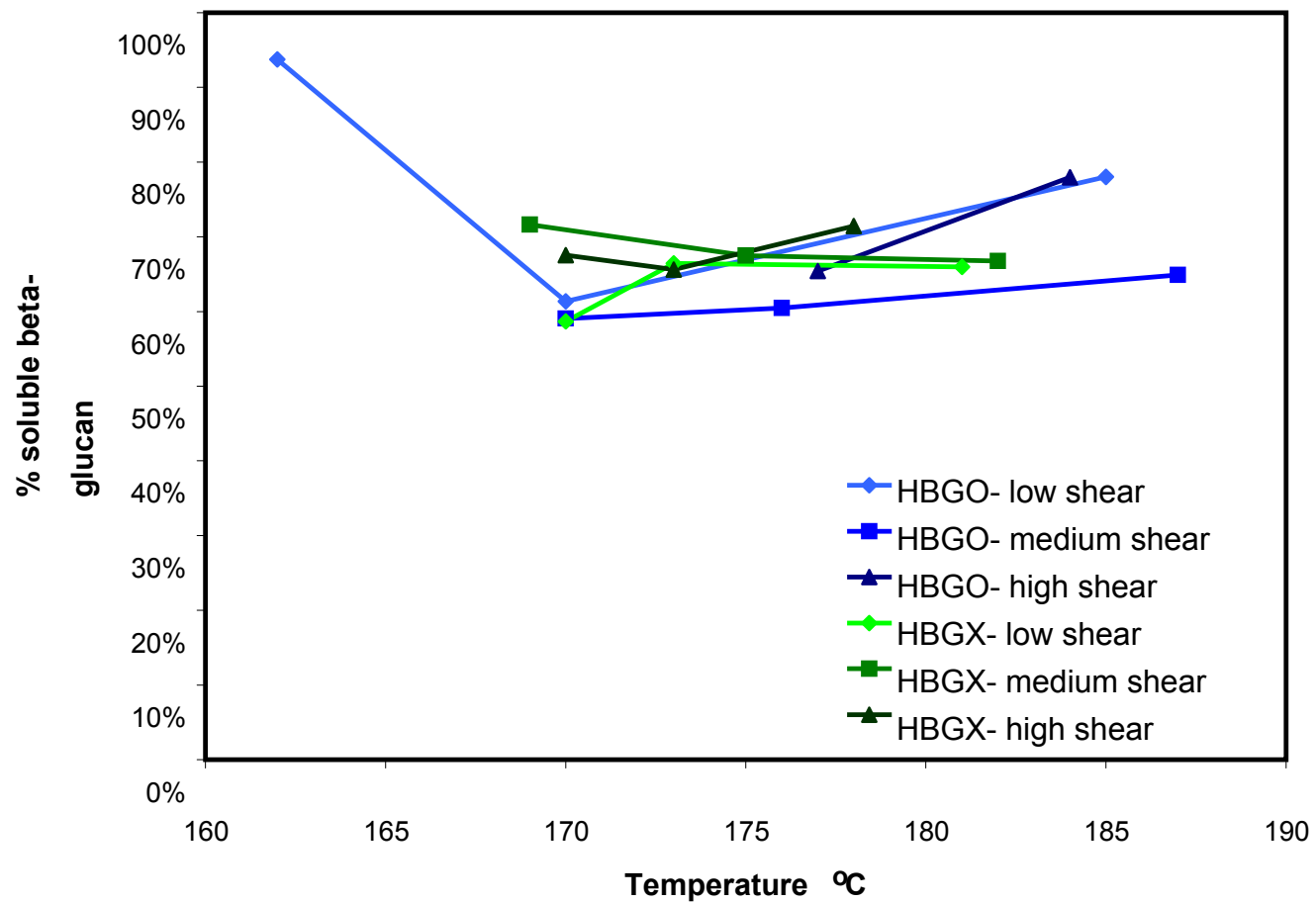
Appendix 3-2 Figure 3 Extract viscosity and % soluble  $\beta$ -glucan in cereals containing different amount of  $\beta$ -glucan



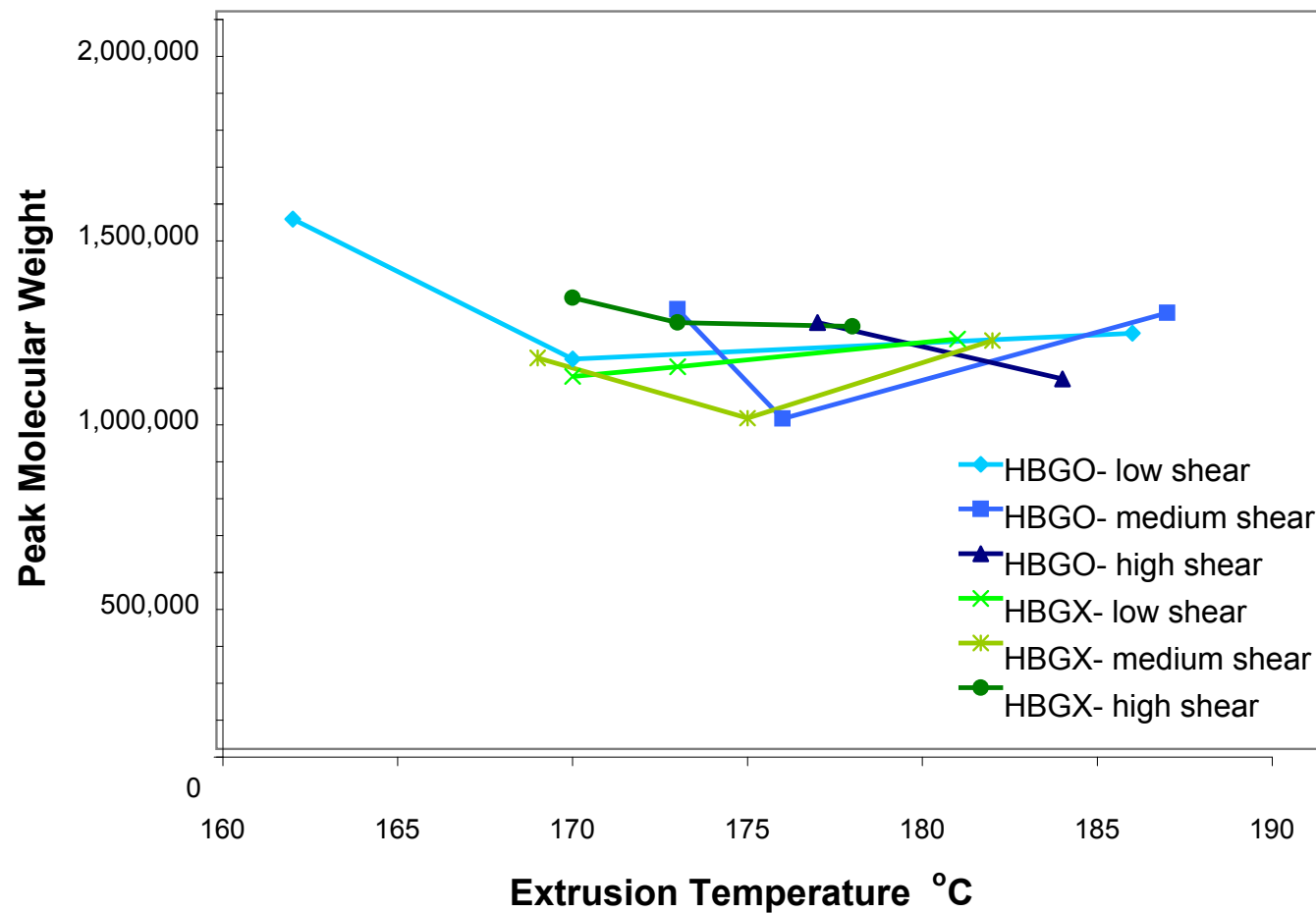
Appendix 3-2 Figure 4: Apparent viscosity of extracts for extruded cereals made with oat bran ingredient (22%  $\beta$ -glucan).



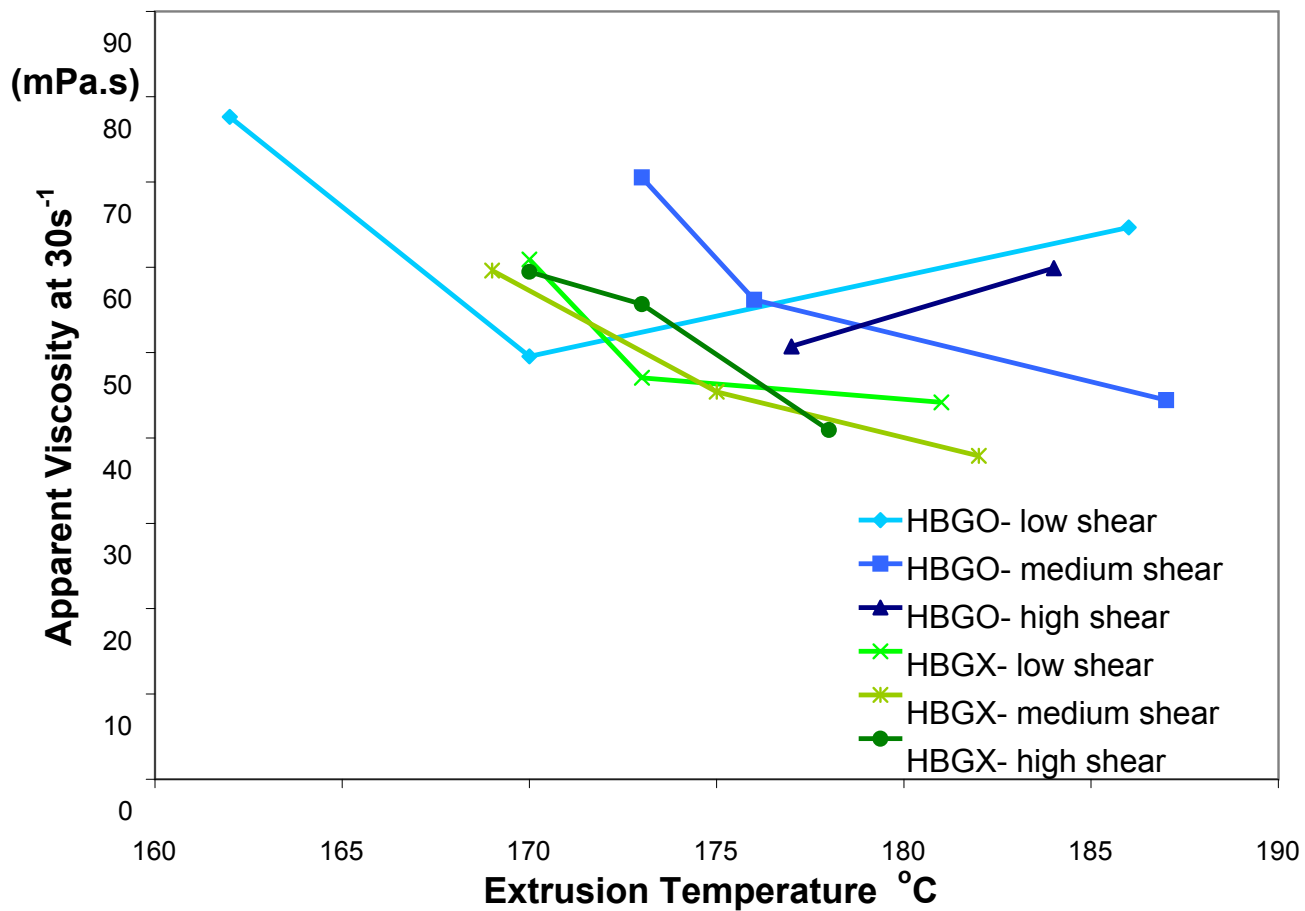
Appendix 3-2 Figure 5: Effect of extrusion temperature and shear on  $\beta$ -glucan solubility



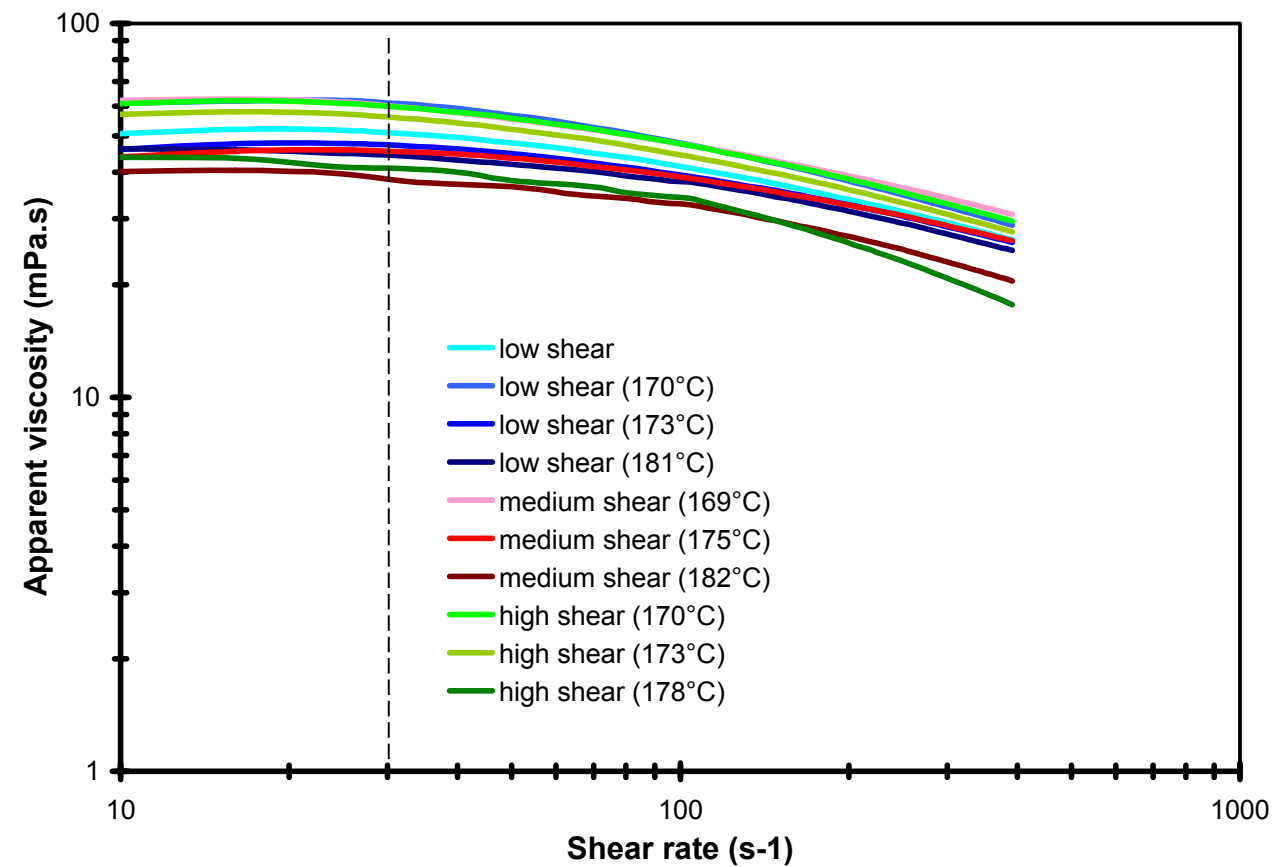
Appendix 3-2 Figure 6: Effect of extrusion temperature and shear rate on molecular weight of extractable  $\beta$ -glucan



Appendix 3-2 Figure 7: Effect of extrusion temperature and shear on extract viscosity.



Appendix 3-2 Figure 8: Apparent viscosity of extracts from extruded cereal made with extracted  $\beta$ -glucan concentrate (HBGX)





## Appendix 5-1

### Ingredient Listing for Study Products

<i>Product</i>	<i>Ingredient Listing</i>
Control Combination RTE Cereal	Wheat, sultanas, apple, sugar
MBG Combination RTE Cereal	Whole oats, whole wheat, oat bran, sugar, honey, glucose, malt extract, salt, flavour, trisodium phosphate, annatto, vitamins and minerals.
HBG Combination RTE Cereal	Oat bran, whole oats, whole wheat, rice, sultanas, dried apple, malt extract (barley), salt, vitamins & minerals, emulsifier (471), preservative (220)*.
Control Porridge	Rice, sultanas, sugar, milk powder
MBG Porridge	Rolled oats, sultanas, sugar, refined oat bran
HBG Porridge	Rolled oats, refined oat bran, sultanas, sugar
Control Muesli Bar	Wholegrain cereals (43%), [Wholegrain wheat flour(30%), Wholegrain rye flour (8%), Wholegrain barley flour (5%), Vitamin E Acetate], Maltodextrin (wheat), Dried Apples (8.0%), Wheat dextrin (soluble dietary fibre), Glucose syrup (wheat), Wheat bran, Sugar, Fructose, Dextrose, Humectants (422, 420), Vegetable fat, Flavours, Emulsifier (471), Antioxidant (Soy) (306), Colours (150d, 160b), Flavours ( Includes Cinnamon Extract), Added sulphites
HBG Muesli Bar	Oat bran, fructose, wheat dextrin, palm olein, rice flour, wheat flour, glycerin, acacia gum, Ca phosphate, degermed corn flour, wheat starch, lactic acid, malt extract, Na phosphate, Na lactate, Ca carbonate, NaCl, apricot 6.8%
Control Cereal Snack	Cereal Grains (Whole Grain Wheat Flour (32.5%) Wheat Flour), Sugar, Extract of Malted Barley and Rice and /or Barley, Milk Powder, Sugar, Fat-Reduced Cocoa Powder, Maltodextrin, Emulsifier (Soya Lecithin)], Fat-Reduced Cocoa Powder, Barley Malt Extract, Skimmed Milk Powder, Palm Oil, Soya Lecithin, Flavouring: (Chocolate and Vanillin), Salt, Vitamins and Minerals.
HBG Cereal Snack	Refined oat bran, maize flour, wheat flour, sugar, maltodextrin, cocoa, salt

## **Appendix 5-2**

### **Product Evaluation**

Subjects were asked to complete the following questionnaire in relation to physical symptoms they associated with the products.

## Study Products Feedback Questionnaire

*The following questions ask you about any positive or negative symptoms you have experienced since starting the study products as part of your weight loss diet. Please answer as honestly and as accurately as you can.*

1. Did you experience any physical symptoms you could relate to the products in the study?

YES

NO

2. How would you describe these symptoms?

a) Flatulence

b) Abdominal Pain

c) Diarrhoea

d) Constipation

e) Other

---

---

---

---

3. How would you describe the intensity of these symptoms?

- a) Severe
- b) Significant
- c) Moderate
- d) Mild
- e) Very Mild

4. Did these symptoms impact on your ability to continue the regular intake of the foods?

YES

NO

Please describe \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

5. Did you experience any positive symptoms from the products?

YES

NO

Please describe your positive symptoms \_\_\_\_\_

---

---

---

---

---

6. Additional Comments (anything that hasn't been addressed in the above questions that you would like to comment on) \_\_\_\_\_

This image shows a blank sheet of white paper with horizontal ruling lines. The lines are evenly spaced and run across the width of the page. There are no margins, text, or other markings on the paper.

Thank You

## Results

1. Did you experience any physical symptoms you could relate to the products in the study?

2. How would you describe these symptoms?

- Flatulence
- Abdominal Pain
- Diarrhoea
- Constipation
- Other

<i>Question 1</i>			<i>Question 2</i>				
	<i>yes</i>	<i>no</i>	<i>flatulence</i>	<i>abdo pain</i>	<i>diarrhoea</i>	<i>constipation</i>	<i>other</i>
<b>Control</b>	44% (7/16)	9/16 (56%)	3/16 (19%)		1/16 (6%)	2/16 (13%)	1/16 (6%)
<b>MBG</b>	11/21 (52%)	10/21 (48%)	10/21 (52%)		2/21 (10%)	1/21 (5%)	1/21 (5%)
<b>HBG</b>	12/19 (63%)	7/19 (37%)	9/19 (47%)	4/19 (21%)	1/19 (5%)	3/19 (16%)	

Other comments in relation to question 2

<b>Q2</b>	
<b>Control</b>	extreme fullness from cereal/porridge in first 10 days, hard to finish amount given
<b>MBG</b>	skin breakouts over face, shoulders, chest and back
	bowels more regular
<b>HBG</b>	flatulence only in first 2 weeks

3. How would you describe the intensity of these symptoms?

- Severe
- Significant
- Moderate
- Mild
- Very Mild

4. Did these symptoms impact on your ability to continue the regular intake of the foods?

<b>Question3</b>						<b>Question 4</b>	
<b>Intensity of Symptoms</b>						<b>Intake Affected</b>	
	<b>severe</b>	<b>significant</b>	<b>moderate</b>	<b>mild</b>	<b>very mild</b>	<b>yes</b>	<b>no</b>
<b>Control</b>		2/16 (13%)	4/16 (25%)				16/16 (100%)
<b>MBG</b>		3/21 (14%)	4/21 (19%)	4/21 (19%)			21/21 (100%)
<b>HBG</b>	1/19 (5%)	5/19 (26%)	2/19 (11%)	3/19 (16%)	1/19 (5%)	1/19 (5%)	18/19 (95%)

Comments in relation to question 4.

<b>HBG</b>	reduced product intake to one per day due to diarrhoea
------------	--------------------------------------------------------

5. Did you experience any positive symptoms from the products?

<b>Question5</b>		
<b>Positive Symptoms</b>		
	<b>yes</b>	<b>no</b>
<b>Control</b>	16/16 (100%)	
<b>MBG</b>	14/21 (67%)	7/21 (33%)
<b>HBG</b>	17/19 (89%)	2/19 (11%)

Comments in relation to question 5.

<b>Q5</b>	
<b>Control</b>	cereal filling and stopped mid-morning snacking; snacks tasty and stopped unhealthy snacking
	eating more regularly without hunger peaks and lows.
	bowels more regular, aided wt loss
	increased energy
	wt control and appetite suppressant
	decreased afternoon snacking
	snacks felt healthy and encouraged healthy eating
	decreased: flatulence and bloating, tiredness, sugar cravings and hunger
	aided wt loss, increased energy, increased feeling of health, tasty products
	cereal filling until morning tea, snacks satisfying and kept hunger pangs away until dinner time
	kept hunger pangs at bay
	increased sense of wellbeing and balanced the body
	portion size perfect
	feeling of satisfaction throughout day, hunger stabilized
	muesli bar helped control afternoon sugar cravings
<b>MBG</b>	not hungry between breakfast and lunch
	more regular bowels, decreased appetite
	weight loss
	reduced snacking on unhealthy foods
	improved appetite at breakfast, bowels more regular, increased fullness
	increased feeling of fullness
	porridge filling
	increased wellbeing
	snacks yummy and satisfying especially at night - helped avoid biscuits etc
	increased energy and decreased knee pain



<b>HBG</b>	products filling, made me feel content
	feeling well and healthier, not as bloated, less abdo pain
	bowels more regular
	products all filling, bowels more regular
	felt fuller for longer
	porridge filling
	encouraged eating cereal
	less bloated, more regular
	breakfast filling for a longer period, museli bars very satisfying
	snack foods healthy, took a while to eat and very filling
	increased energy
	regulated eating, products convenient and easy to carry
	decreased hunger
	regulated bowels as before starting the products had irritable bowel and could go up to 5 times per day but with products this reduced to once a day; breast soreness before period significantly reduced

6. Additional Comments (anything that hasn't been addressed in the above questions that you would like to comment on)

<b>Q6</b>	
<b>Control</b>	set snacks and portion control helped avoid overeating unhealthy foods
<b>MBG</b>	study improved eating habits and overall health
<b>HBG</b>	negative symptoms moderate for first 6 weeks but then settled down
	decreased overall sugar intake and its associated cravings
	regulated appetite, increased water intake due to products, more sensitive to commercial products with high sugar or salt intakes

## **Product Evaluation Questionnaires and Results**

The questionnaires and results contained in this appendix were designed by Uncle Tobys staff at Rutherglen, Victoria, Australia.

## **Product Evaluation**

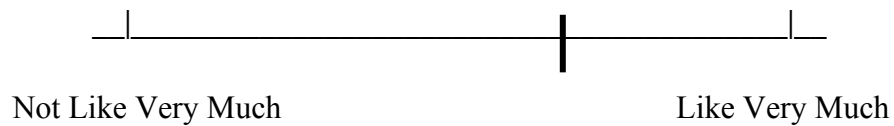
**BG – Group**

**1**

**You have been involved in a study and we would like to know what you think about the products that you have been eating.**

**For questions that appear like this could you please place a small vertical line on the scale where you believe it should be. Remember there are no right or wrong answers. We just want to know what YOU think. Thankyou!**

Eg




---

## **Breakfast**

**You had the opportunity to taste both breakfast options, could you please tell us what you thought about them.**

**COMBINATION CEREAL – SAMPLE NO. 705**

1. **OVERALL**, How much did you **LIKE** or **DISLIKE** this cereal?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

2. How much did you **LIKE** or **DISLIKE** the **APPEARANCE** of the cereal?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

3. How much did you **LIKE** or **DISLIKE** the **FLAVOUR** of this cereal?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

- 4a. How **WEAK** or **STRONG** do you think the **FLAVOUR** of this cereal is?

*(Please indicate by placing a small vertical mark on the line **below and label it 'a'** )*

\_\_\_\_\_

Very Weak

Very Strong

- 4b. If we were making this cereal perfect for you, how **WEAK** or **STRONG** would the **FLAVOUR** be? *(Please indicate by placing a small vertical mark on the line above and labeling it 'b')*

5. How much do you **LIKE** or **DISLIKE** the **TEXTURE** of this cereal?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

6. How much do you **LIKE** or **DISLIKE** the **CRUNCHINESS** of this cereal?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

- 7a. How **CRUNCHY** or **NOT CRUNCHY** do you think this cereal is?

*(Please indicate by placing a small vertical mark on the line below and label it 'a' )*

\_\_\_\_\_

Not Crunchy

Very Crunchy

- 7b. If we were making this cereal perfect for you, how **CRUNCHY** would it be?

*(Please indicate by placing a small vertical mark on the line above and labeling it 'b')*

8. How satisfying/ filling was the product after you had eaten it?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Not Very filling

Very Filling

9. If you were able to buy this product from your local grocery store at a reasonable price how **LIKELY** would you be **to BUY** this Cereal?

*(Please tick one.)*

Definitely Would Buy

☐

Probably Would Buy

☐

Unsure

☐

Probably Wouldn't Buy

☐

Definitely Wouldn't Buy

☐

**PORRIDGE – SAMPLE NO. 706**

1. **OVERALL**, How much did you **LIKE** or **DISLIKE** the porridge?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

2. How much did you **LIKE** or **DISLIKE** the **APPEARANCE** of the porridge?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

3. How much did you **LIKE** or **DISLIKE** the **FLAVOUR** of this porridge?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

- 4a. How **WEAK** or **STRONG** do you think the **FLAVOUR** of this porridge is?

*(Please indicate by placing a small vertical mark on the line **below and label it 'a'** )*

\_\_\_\_\_

Very Weak

Very Strong

- 4b. If we were making this porridge perfect for you, how **WEAK** or **STRONG** would the **FLAVOUR** be? *(Please indicate by placing a small vertical mark on the line above and label it 'b')*

5. How much do you **LIKE** or **DISLIKE** the **TEXTURE** of this porridge?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

6. How much do you **LIKE** or **DISLIKE** the **THICKNESS** of this porridge?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much



7. How satisfying/ filling was the product after you had eaten it?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Not Very filling

Very Filling

8. If you were able to buy this product from your local grocery store at a reasonable price how **LIKELY** would you be **to BUY** this Porridge?

*(Please tick one.)*

Definitely Would Buy

☐

Probably Would Buy

☐

Unsure

☐

Probably Wouldn't Buy

☐

Definitely Wouldn't Buy

☐

**SNACKS****CEREAL BAR – SAMPLE NO. 707**

1. **OVERALL**, How much did you **LIKE** or **DISLIKE** this cereal bar?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

2. How much did you **LIKE** or **DISLIKE** the **APPEARANCE** of the cereal bar?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

3. How much did you **LIKE** or **DISLIKE** the **FLAVOUR** of this cereal bar?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

- 4a How **WEAK** or **STRONG** do you think the **FLAVOUR** of this cereal bar is?

*(Please indicate by placing a small vertical mark on the line **below and label it 'a'** )*

\_\_\_\_\_

Very Weak

Very Strong

- 4b. If we were making this cereal bar perfect for you, how **WEAK** or **STRONG** would the **FLAVOUR** be? *(Please indicate by placing a small vertical mark on the line above and label it 'b')*

5. How much do you **LIKE** or **DISLIKE** the **TEXTURE** of this cereal bar?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

6. How satisfying/ filling was the **CEREAL BAR** after you had eaten it?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Not Very filling

Very Filling

7. If you were able to buy this product from your local grocery store how **LIKELY** would you be **to BUY** this Cereal Bar?

*(Please tick one.)*

Definitely Would Buy

☐

Probably Would Buy

☐

Unsure

☐

Probably Wouldn't Buy

☐

Definitely Wouldn't Buy

☐

**CHOC CEREAL SNACK – SAMPLE NO. 708**

1. **OVERALL**, How much did you **LIKE** or **DISLIKE** this cereal snack?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

2. How much did you **LIKE** or **DISLIKE** the **APPEARANCE** of the cereal snack?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

3. How much did you **LIKE** or **DISLIKE** the **FLAVOUR** of this cereal snack?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

- 4a. How **WEAK** or **STRONG** do you think the **FLAVOUR** of this cereal snack is?

*(Please indicate by placing a small vertical mark on the line **below and label it 'a'** )*

\_\_\_\_\_

Very Weak

Very Strong

- 4b. If we were making this cereal snack perfect for you, how **WEAK** or **STRONG** would the **FLAVOUR** be? *(Please indicate by placing a small vertical mark on the line above and label it 'b')*

5. How much do you **LIKE** or **DISLIKE** the **TEXTURE** of this cereal snack?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

6. How much do you **LIKE** or **DISLIKE** the **CRUNCHINESS** of this cereal snack?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

- 7a. How **CRUNCHY** or **NOT CRUNCHY** do you think this cereal snack is?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Not Crunchy

Very Crunchy

- 7b. If we were making this cereal snack perfect for you, how **CRUNCHY** would it be?

*(Please indicate by placing an I on the line **above**)*

8. How satisfying/ filling was the product after you had eaten it?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Not Very filling

Very Filling

9. If you were able to buy this product from your local grocery store how **LIKELY** would you be **to BUY** this Cereal Snack?

*(Please tick one.)*

Definitely Would Buy

☐

Probably Would Buy

☐

Unsure

☐

Probably Wouldn't Buy

☐

Definitely Wouldn't Buy

☐

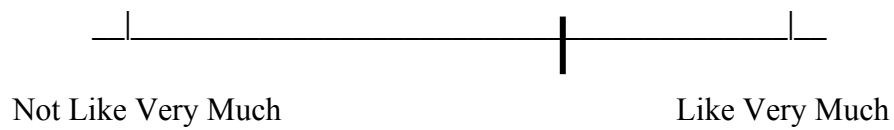
## **Product Evaluation**

**BG – Group 2**

**You have been involved in a study and we would like to know what you think about the products that you have been eating.**

**For questions that appear like this could you please place a small vertical line on the scale where you believe it should be. Remember there are no right or wrong answers. We just want to know what YOU think. Thankyou!**

Eg



## **Breakfast**

**You had the opportunity to taste both breakfast cereals, could you please tell us what you thought about them.**



**COMBINATION CEREAL – SAMPLE NO. 905**

1. **OVERALL**, How much did you **LIKE** or **DISLIKE** this cereal?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

2. How much did you **LIKE** or **DISLIKE** the **APPEARANCE** of the cereal?

*(Please indicate by placing a small vertical mark on the line below)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

3. How much did you **LIKE** or **DISLIKE** the **FLAVOUR** of this cereal?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

- 4a. How **WEAK** or **STRONG** do you think the **FLAVOUR** of this cereal is?

*(Please indicate by placing a small vertical mark on the line **below and label it 'a'** )*

\_\_\_\_\_

Very Weak

Very Strong

- 4b. If we were making this cereal perfect for you. How **WEAK** or **STRONG** would the **FLAVOUR** be? *(Please indicate by placing a small vertical mark on the line above and labeling it 'b')*

5. How much do you **LIKE** or **DISLIKE** the **TEXTURE** of this cereal?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

6. How much do you **LIKE** or **DISLIKE** the **CRUNCHINESS** of this cereal?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

- 7a. How **CRUNCHY** or **NOT CRUNCHY** do you think this cereal is?

*(Please indicate by placing a small vertical mark on the line **below and label it 'a'** )*

\_\_\_\_\_

Not Crunchy

Very Crunchy

- 7b. If we were making this cereal perfect for you. How **CRUNCHY** would it be?

*(Please indicate by placing a small vertical mark on the line above and labeling it 'b')*

8. How satisfying/ filling was the product after you had eaten it?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Not Very filling

Very Filling

9. If you were able to buy this product from your local grocery store at a reasonable price how **LIKELY** would you be **to BUY** this Cereal?

*(Please tick one.)*

Definitely Would Buy

☐

Probably Would Buy

☐

Unsure

☐

Probably Wouldn't Buy

☐

Definitely Wouldn't Buy

☐

**PORRIDGE – SAMPLE NO. 906**

1. **OVERALL**, How much did you **LIKE** or **DISLIKE** the porridge?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

2. How much did you **LIKE** or **DISLIKE** the **APPEARANCE** of the porridge?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

3. How much did you **LIKE** or **DISLIKE** the **FLAVOUR** of this porridge?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

- 4a. How **WEAK** or **STRONG** do you think the **FLAVOUR** of this porridge is?

*(Please indicate by placing a small vertical mark on the line **below and label it 'a'** )*

\_\_\_\_\_

Very Weak

Very Strong

- 4b. If we were making this porridge perfect for you. How **WEAK** or **STRONG** would the **FLAVOUR** be? *(Please indicate by placing a small vertical mark on the line above and label it 'b')*

5. How much do you **LIKE** or **DISLIKE** the **TEXTURE** of this porridge?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

6. How much do you **LIKE** or **DISLIKE** the **THICKNESS** of this porridge?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

7. How satisfying/ filling was the product after you had eaten it?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Not Very filling

Very Filling

8. If you were able to buy this product from your local grocery store at a reasonable price how **LIKELY** would you be **to BUY** this Porridge?

*(Please tick one.)*

Definitely Would Buy

☐

Probably Would Buy

☐

Unsure

☐

Probably Wouldn't Buy

☐

Definitely Wouldn't Buy

☐

## SNACKS

### CEREAL BAR – SAMPLE NO. 707

1. **OVERALL**, How much did you **LIKE** or **DISLIKE** this cereal bar?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

2. How much did you **LIKE** or **DISLIKE** the **APPEARANCE** of the cereal bar?

*(Please indicate by placing a small vertical mark on the line below)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

3. How much did you **LIKE** or **DISLIKE** the **FLAVOUR** of this cerealbar?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

- 4a How **WEAK** or **STRONG** do you think the **FLAVOUR** of this cereal bar is?

*(Please indicate by placing a small vertical mark on the line **below and label it 'a'** )*

\_\_\_\_\_

Very Weak

Very Strong

- 4b. If we were making this cereal bar perfect for you. How **WEAK** or **STRONG** would the **FLAVOUR** be? *(Please indicate by placing a small vertical mark on the line above and label it 'b')*

5. How much do you **LIKE** or **DISLIKE** the **TEXTURE** of this cereal bar?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

6. How satisfying/ filling was the **CEREAL BAR** after you had eaten it?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Not Very filling

Very Filling

7. If you were able to buy this product from your local grocery store how **LIKELY** would you be **to BUY** this Cereal Bar?

*(Please tick one.)*

Definitely Would Buy

☐

Probably Would Buy

☐

Unsure

☐

Probably Wouldn't Buy

☐

Definitely Wouldn't Buy

☐



**SNACKS****CEREAL BAR – SAMPLE NO. 507**

1. **OVERALL**, How much did you **LIKE** or **DISLIKE** this cereal bar?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

2. How much did you **LIKE** or **DISLIKE** the **APPEARANCE** of the cereal bar?

*(Please indicate by placing a small vertical mark on the line below)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

3. How much did you **LIKE** or **DISLIKE** the **FLAVOUR** of this cerealbar?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

- 4a How **WEAK** or **STRONG** do you think the **FLAVOUR** of this cereal bar is?

*(Please indicate by placing a small vertical mark on the line **below and label it 'a'** )*

\_\_\_\_\_

Very Weak

Very Strong

- 4b. If we were making this cereal bar perfect for you. How **WEAK** or **STRONG** would the **FLAVOUR** be? *(Please indicate by placing a small vertical mark on the line above and label it 'b')*

5. How much do you **LIKE** or **DISLIKE** the **TEXTURE** of this cereal bar?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

6. How satisfying/ filling was the **CEREAL BAR** after you had eaten it?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Not Very filling

Very Filling

7. If you were able to buy this product from your local grocery store how **LIKELY** would you be **to BUY** this Cereal Bar?

*(Please tick one.)*

Definitely Would Buy

☐

Probably Would Buy

☐

Unsure

☐

Probably Wouldn't Buy

☐

Definitely Wouldn't Buy

☐

**CHOC CEREAL SNACK – SAMPLE NO. 708**

1. **OVERALL**, How much did you **LIKE** or **DISLIKE** this cereal snack?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

2. How much did you **LIKE** or **DISLIKE** the **APPEARANCE** of the cereal snack?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

3. How much did you **LIKE** or **DISLIKE** the **FLAVOUR** of this cereal snack?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

- 4a. How **WEAK** or **STRONG** do you think the **FLAVOUR** of this cereal snack is?

*(Please indicate by placing a small vertical mark on the line **below and label it 'a'** )*

\_\_\_\_\_

Very Weak

Very Strong

- 4b. If we were making this cereal snack perfect for you. How **WEAK** or **STRONG** would the **FLAVOUR** be? *(Please indicate by placing a small vertical mark on the line above and label it 'b')*

5. How much do you **LIKE** or **DISLIKE** the **TEXTURE** of this cereal snack?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

6. How much do you **LIKE** or **DISLIKE** the **CRUNCHINESS** of this cereal snack?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

- 7a. How **CRUNCHY** or **NOT CRUNCHY** do you think this cereal snack is?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Not Crunchy

Very Crunchy

- 7b. If we were making this cereal snack perfect for you. How **CRUNCHY** would it be?

*(Please indicate by placing an I on the line **above**)*

8. How satisfying/ filling was the product after you had eaten it?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Not Very filling

Very Filling

9. If you were able to buy this product from your local grocery store how **LIKELY** would you be **to BUY** this Cereal Snack?

*(Please tick one.)*

Definitely Would Buy

☐

Probably Would Buy

☐

Unsure

☐

Probably Wouldn't Buy

☐

Definitely Wouldn't Buy

☐


---

**CHOC CEREAL SNACK – SAMPLE NO. 508**

1. **OVERALL**, How much did you **LIKE** or **DISLIKE** this cereal snack?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

2. How much did you **LIKE** or **DISLIKE** the **APPEARANCE** of the cereal snack?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

3. How much did you **LIKE** or **DISLIKE** the **FLAVOUR** of this cereal snack?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

- 4a. How **WEAK** or **STRONG** do you think the **FLAVOUR** of this cereal snack is?

*(Please indicate by placing a small vertical mark on the line **below and label it 'a'** )*

\_\_\_\_\_

Very Weak

Very Strong

- 4b. If we were making this cereal snack perfect for you. How **WEAK** or **STRONG** would the **FLAVOUR** be? *(Please indicate by placing a small vertical mark on the line above and label it 'b')*

5. How much do you **LIKE** or **DISLIKE** the **TEXTURE** of this cereal snack?  
*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

6. How much do you **LIKE** or **DISLIKE** the **CRUNCHINESS** of this cereal snack?  
*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Dislike Very Much

Like Very Much

- 7a. How **CRUNCHY** or **NOT CRUNCHY** do you think this cereal snack is?  
*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Not Crunchy

Very Crunchy

- 7b. If we were making this cereal snack perfect for you. How **CRUNCHY** would it be?  
*(Please indicate by placing an I on the line **above**)*



8. How satisfying/ filling was the product after you had eaten it?

*(Please indicate by placing a small vertical mark on the line **below**)*

\_\_\_\_\_

Not Very filling

Very Filling

9. If you were able to buy this product from your local grocery store how **LIKELY** would you be **to BUY** this Cereal Snack?

*(Please tick one.)*

Definitely Would Buy

☐

Probably Would Buy

☐

Unsure

☐

Probably Wouldn't Buy

☐

Definitely Wouldn't Buy

☐

## Results

