

Chickpeas and Human Health

The effect of chickpea consumption on
some physiological and metabolic
parameters.

by

Jane Pittaway BBiomed Sci (Hons)

Submitted in fulfilment of the
requirements for the degree of

Masters by Research in Biomedical Sciences

University of Tasmania, August 2006

Declaration of Originality

This thesis contains no material that has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis. To the best of my knowledge and belief, no material previously published or written by another person except where due acknowledgement is made appears in the text of the thesis [RHD Resource Book 2003; Appendix A.3: 10(4) (b), p 40].

Jane Kneller Pittaway

Statement of Authority of Access

This thesis may be made available for loan and limited copying in accordance with the Copyright Act 1968 (RHD Resource Book 2003; Thesis preparation p23).

Jane Kneller Pittaway

List of Abbreviations and Glossary

AIHW	Australian Institute of Health and Welfare
PAR	Population Attributable Risk
%E	Percentage of Energy
%TF	Percentage of Total Fat
BTT	Bowel Transit Time
C:I	Carbohydrate to Insulin ratio
CHD	Coronary Heart Disease
CVD	Cardiovascular Diseases
g	grams
GI	Glycaemic Index
GIT	Gastrointestinal Tract
GL	Glycaemic Load
HCHF	High Carbohydrate High Fibre
HDL-C	High Density Lipoprotein Cholesterol
HID	Hypercholesterolaemia Inducing Diet
HL	High Leguminous diet
HMG CoA-reductase	3-hydroxy-3-methylglutaryl coenzyme A reductase
HOMA-IR	Homeostasis Model Assessment of Insulin Resistance
IDDM	Insulin Dependent Diabetes Mellitus
kg	kilograms
LC	Low Carbohydrate diet
LCLF	Low Carbohydrate Low Fibre

LDL-C	Low Density Lipoprotein Cholesterol
LKF	Lentil Kernel Fibre
mcg	micrograms
mg	milligrams
MJ	Mega Joules
MUFA	Monounsaturated Fatty Acids
NHANES 1	First National Health and Nutrition Examination Survey
NHEFS	First National Health and Nutrition Examination Survey Epidemiologic Follow-up Study
NIDDM	Non-Insulin Dependent Diabetes Mellitus
NSP	Non Starch Polysaccharide
P:M:S ratio	Ratio of polyunsaturated to monounsaturated to saturated fatty acids
P:S ratio	Ratio of Polyunsaturated to Saturated fatty acids
PUFA	Polyunsaturated Fatty Acids
SEM	Standard Error of the Mean
SFA	Saturated Fatty Acids
TC	Total Cholesterol
USDA	United States (of America) Department of Agriculture
VLDL-C	Very Low Density Lipoprotein Cholesterol
WHO	World Health Organisation
Satiation	Degree of fullness leading to meal cessation
Satiety	Interval between cessation of one meal and initiation of the next

Thesis Abstract

Pulses (legumes) are a common dietary constituent of ethnic communities exhibiting lower rates of cardiovascular disease (CVD). The following studies examined the effect of including chickpeas in an 'Australian' diet on CVD risk factors. Participants were free-living volunteers aged 30 to 70 years.

Study 1 investigated the effect of chickpeas on serum lipids, lipoproteins, glycaemic control, bowel function and satiation (degree of fullness leading to meal cessation) compared to a higher-fibre wheat-supplemented diet (Chapter 2). Participants completed two controlled dietary interventions (chickpea-supplemented and higher-fibre wheat-supplemented), isocaloric with their usual dietary intake, in random order. The design of the intervention diets was for matched macronutrient content and dietary fibre however increased consumption of polyunsaturated fatty acids (PUFA) during the chickpea-supplemented diet was noted. Small but significant reductions in mean serum total cholesterol and low density lipoprotein-cholesterol (LDL-C) were reported following the chickpea diet compared to the wheat. Statistical analysis suggested a relationship between increased consumption of PUFA and reduction in cholesterol during the chickpea intervention but could not discern the source of PUFA. Chickpea supplementation did not adversely affect bowel function and participants found them very satiating. There was no effect on glycaemic control. A small, sub-study compared the effects of an isocaloric, lower-fibre wheat

diet to the higher-fibre wheat, to evaluate the effect of quantity of fibre as well as source on bowel health and satiety. During the lower-fibre wheat intervention, some participants reported lower satiation, and poorer bowel health.

Some of the results from this study were included in a larger, collaborative study investigating the effect of chickpeas on serum lipids and lipoproteins in two centres, Launceston and Melbourne. The Melbourne group followed a similar controlled, random crossover comparison of a chickpea-supplemented diet to a higher-fibre wheat-supplemented diet, also endeavouring to match macronutrient content and dietary fibre. The Melbourne group also reported small but significant reductions in mean serum LDL- and total cholesterol but reported discrepancies in consumption of PUFA as well as dietary fibre between the intervention diets. Statistical analysis of the combined results suggested a relationship between increased consumption of PUFA and dietary fibre and a reduction in cholesterol during the chickpea intervention. Appendix 1 is a description of this collaborative study, formatted as a scientific paper, accepted for publication.

Study 2 investigated whether results from the controlled study would translate to *ad libitum* situations (Chapter 3). The study followed an ordered crossover design where participants followed their habitual *ad libitum* dietary intake for four weeks (familiarisation phase), incorporated a minimum of four 300g (net weight) cans of chickpeas per week for 12

weeks and then resumed their habitual diet for another four weeks (usual phase). Small but significant reductions in body weight, body mass index (BMI), serum TC, fasting insulin and HOMA-IR occurred following the chickpea phase, compared to the post-chickpea usual phase. Results suggested that participants positively altered their eating pattern during the pre-chickpea familiarisation phase, sustained these changes during the 12-week chickpea phase but regressed during the usual phase. Participants consumed significantly more dietary fibre and PUFA during the chickpea phase and less total fat and saturated fatty acids (SFA) compared to the usual phase. Perceived bowel health remained constant throughout the study, while satiation increased significantly during the chickpea phase along with a small but significant reduction in mean body weight.

Incorporating chickpeas into an 'Australian' style diet resulted in increased consumption of PUFA and dietary fibre that produced small but significant reductions in serum TC, BMI and glycaemic control, high satiation and little effect on bowel function. Individuals wishing to reduce CVD risk may choose to include chickpeas in their diet.

Thesis Acknowledgements

I gratefully acknowledge the generosity of the Clifford Craig Medical Research Trust and the Northern Tasmania Pathology Service for the use of their facilities. The Grains Research and Development Corporation (Australia) (GRDC) provided funding for the controlled studies (Ch 2 & App 1) and Simplot (Australia) donated 2,400 cans of chickpeas for the *ad libitum* study (Ch 3). I would also like to acknowledge my supervisor, Professor Madeleine Ball, for her timely advice and guidance and biostatistician Dr. Iain Robertson for statistical and analytical advice. My colleague, Kiran Ahuja, assisted in the recruitment and interview of participants in the controlled study and in the creation of the intervention diets for the same study. She has also been an invaluable source of encouragement and emotional support. Catherine Murty assisted with processing of dietary data and food group analysis for the *ad libitum* study. I would like to thank members of the public of Northern Tasmania for their enthusiastic response to calls for volunteers to participate in this project. In particular, I would like to extend my appreciation to the 72 participants who were able to complete either the controlled or the *ad libitum* study. Finally, I could not have attempted this work without the understanding, encouragement and support of my husband, Michael Bok and our three daughters, Zoe, Nina and Erin.

Table of Contents

Declaration of Originality	ii
List of Abbreviations and Glossary	iii
Thesis Abstract.....	v
Thesis Acknowledgements.....	viii
Journal articles and presentations to learned societies arising from the work described in this thesis.	xii

Chapter 1

General Introduction.....	1
1.1 The diet-heart hypothesis	2
1.2 Legumes and pulses	6
1.3 Chickpeas.....	28
1.4 Aim of studies	41
1.5 Design of studies.....	43

Chapter 2

Effects of a Controlled Diet Supplemented with Chickpeas on Serum Lipids, Glucose Tolerance, Satiation and Bowel Function	48
2.1 Abstract	48
2.2 Introduction.....	49
2.3 Materials and Methods	51
2.4 Results	55
2.5 Discussion	63
2.6 Conclusion.....	67

Chapter 3

Chickpeas influence P:S ratio and fibre content of ad libitum dietary intake leading to improved serum lipid profile, glycaemic control and satiation.	69
3.1 Abstract	69
3.2 Introduction.....	70
3.3 Method	72
3.4 Results	77
3.5 Discussion	88
3.6 Summary and Conclusion	91

Chapter 4

General Discussion	93
---------------------------------	-----------

Appendix 1

Dietary supplementation with chickpeas for at least five weeks results in small but significant reductions in serum total- and LDL-cholesterol in adult women and men.	101
--	------------

Appendix 2

Abstracts and Posters from Conference Presentations.....	118
References.....	122

List of Tables and Figures

Chapter 1

Table 1.1 Selected nutrient content per 100g dry raw weight of soybeans and chickpeas (legumes) compared to brown rice and wheat (cereals).	8
--	---

Chapter 2

Table 2.1 Comparison of nutritional intake and bodyweight at the end of the dietary periods	56
Table 2.2 Comparison of background nutritional intake (apart from chickpea and wheat products) at the end of the dietary periods	58
Table 2.3 Comparison of results for each dietary intervention phase	59
Fig 2 1. Comparison of bowel transit times between the chickpea, wheat and low-fibre wheat diets for each participant	61

Chapter 3

Table 3.1. Baseline characteristics of the study participants.....	78
Table 3.2 Dietary intake during the final week of the familiarisation, chickpea and usual dietary phases	79
Table 3.3. Mean difference in dietary components consumed in the first and final weeks of the chickpea and usual dietary phases	81
Table 3.4. Mean difference in anthropometric and laboratory measurements recorded at the beginning and end of each dietary phase	83
Table 3.5. Individual effect of Usual versus Chickpea dietary phases and dietary components on serum TC and insulin	85
Table 3.6. Mean difference in bowel function and satiation measured during the first and final weeks of the chickpea and usual phases	87

Journal articles and presentations to learned societies arising from the work described in this thesis.¹

Articles accepted for publication

J.K. Pittaway, K.D.K. Ahuja, I.K. Robertson, P.J. Nestel, M.J. Ball:

Dietary supplementation with chickpeas for at least five weeks results in small but significant reductions in serum total- and LDL-cholesterol in adult women and men (Appendix 1).

- Accepted by Archives of Nutrition and Metabolism June 2006.

Jane K. Pittaway, BBiomedSc(Hons), Kiran D. K. Ahuja, MBiomedSc, Iain K. Robertson, MMedSci, Madeleine J. Ball, FRCPath:

Effects of a Controlled Diet Supplemented with Chickpeas on Serum Lipids, Glucose Tolerance, Satiety and Bowel Function (Chapter 2).

- Accepted by Journal of the American College of Nutrition July 2006.

¹ All contributors were involved in study design, protocol and revision of manuscript. JKP wrote the original manuscripts and edited subsequent versions; MJB: Investigator in charge and approved final manuscript; IKR: Consultant biostatistician; JKP: Administered and conducted the studies, statistical and laboratory analyst; KDKA: Assisted in data collection, laboratory testing and statistical analysis; PJN: Investigator in charge of Melbourne group.

Presentations to learned societies

Pittaway, JK, Ahuja, KD, Chronopoulos, A, Cehun, M, Robertson, IK, Nestel, PJ, Ball, MJ:

The Effect of Chickpeas on Human Serum Lipids and Lipoproteins.

Presented as a poster at the 2004 Nutrition Society of Australia 28th Annual Scientific Meeting, Brisbane, Queensland, Australia, in conjunction with the Nutrition Society of New Zealand and the International Council of Clinical Nutrition, August 11th-13th 2004. (Appendix 2)

- Abstract published in Asia Pac J Clin Nutr 13:S70; 2004.

JK Pittaway, KDK Ahuja, IK Robertson and MJ Ball:

Effects of a Controlled Diet Supplemented with Chickpeas on Serum Lipids, Glucose Tolerance, Satiety and Bowel Function

To be presented as a poster at the 2006 Nutrition Society of Australia 30th Annual Scientific Meeting, Sydney, NSW, Australia, December 2006.

JK Pittaway, IK Robertson, MJ Ball

Chickpeas influence P:S ratio and fibre content of ad libitum dietary intake leading to improved serum lipid profile, glycaemic control and satiation

To be presented as a poster at the 2006 Nutrition Society of Australia 30th Annual Scientific Meeting, Sydney, NSW, Australia, December 2006.

Chapter 1

General Introduction

Worldwide, the rate of increase in chronic, non-communicable diseases such as cardiovascular disease (CVD), diabetes, obesity, hypertension and some cancers is fast outstripping that of communicable infectious diseases. In 2001, 60% of global mortality and 46% morbidity were due to non-communicable diseases (182), CVD in particular. While age standardised death rates in many of the wealthier countries have fallen from their peak of the 1950's and 1960's, rates in many developing countries are now on the rise – especially in Central Europe (2, 104), India (131) and China (182). The hypotheses advanced for the increases in CVD in these countries cite changing dietary, exercise and lifestyle patterns partly related to changing socioeconomic circumstances of the populations (2, 104). In some countries during the last decade, prevalence of obesity has tripled (182). The forecast is that by 2020, 75% of global deaths will be due to non-communicable diseases and 75% of those will occur in developing countries (182).

The INTERHEART Study (189) examined if modifiable factors associated with development of CVD in America and Western European countries, were present to the same degree (population attributable risk – PAR) in other countries and ethnic populations. It was found that the following nine factors accounted for 90% of PAR in men and 94% in women, regardless of age or ethnicity: abnormal lipid profile, tobacco smoking, psychosocial factors (financial-, social-, or employment–related stress), abdominal obesity,

hypertension, lack of daily consumption of fruit and vegetables, lack of regular physical activity, diabetes and alcohol consumption more than three days per week. Seven of the nine factors have a diet-related association.

1.1 The diet-heart hypothesis

The diet-heart hypothesis suggests that differences in dietary habit, rather than racial or genetic differences, are responsible for the variation in CVD mortality rates between ethnic populations (12, 23, 66, 77). A number of epidemiological studies conducted in the 1950's and 60's such as the Ni-Hon-San study (140 356), the Ireland-Boston Diet-Heart Study (89 43) and the Seven Countries Study (111, 176) demonstrated this. In the early 1970's, Burkitt and Trowell examined the link between the rate of 'non-infective' diseases - such as coronary heart disease (CHD), bowel disease, obesity and diabetes, and changes to the traditional diets of urban-dwelling Africans who had adopted a more 'Western' diet and lifestyle (23, 169). It was found that the effect of dietary fibre on the gastro-intestinal tract was related to caloric intake, bowel health and serum total cholesterol concentrations.

A consequence of these early food pattern studies has been the interest shown in traditional world diets; in particular, the Mediterranean Diet and the traditional Japanese diet (88, 152). Both diets contain large amounts of unprocessed plant-based foods and very little saturated fat or animal-based products. Pulses are the common factor in these and other traditional diets, consumed in conjunction with cereals or tubers to provide essential nutrients, unsaturated fat, protein and dietary fibre (134). Dietary intervention studies

such as the Indian Diet Heart Study (156) and the Lyon Diet Heart Study (33) have also supported the health promoting nature of intervention diets based on beneficial traditional dietary patterns. These patterns include increased consumption of dietary fibre, resistant starch, plant protein and unsaturated fats (MUFA and PUFA) along with reduced consumption of animal protein and saturated fats. In 1990, the World Health Organisation Expert Committee on Diet, Nutrition and Prevention of Chronic Diseases, taking heed of outcomes regarding comparison of world food patterns, recommended for the first time a goal of consuming 400 g/day of fresh fruit and vegetables and 30 g/day of pulses (70, 120, 150, 156).

1.1.1 Dietary Fat, Carbohydrate and CVD Risk Factors

Various studies have reported that compared to dietary carbohydrate, dietary saturated and unsaturated fatty acids increase high-density lipoprotein cholesterol (HDL-C) (65, 86, 112). In addition, most dietary saturated fatty acids (SFA) and trans unsaturated fatty acids increase serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) concentrations, cis unsaturated fatty acids reduced serum TC and LDL-C and stearic acid has little or no effect (65, 86, 112, 183). Furthermore, research suggests substitution of unsaturated fatty acids for SFA may also improve insulin sensitivity (66, 67, 148, 183). Early controlled feeding studies led to predictive equations such as that of Keys et al (78) and Hegsted et al (57), from which the P:S ratio emerged as a key dietary influence on serum TC concentration (52, 86). The P:S ratio (and subsequent P:M:S ratio) provides some acknowledgment of in vivo interaction between the three classes of

fatty acids which has confounded many studies i.e. whether the observed effect is due to an increase in dietary (cis) polyunsaturated fatty acids (PUFA) and/or monounsaturated fatty acids (MUFA) or the replacement of SFA (or trans unsaturated fatty acids) (86, 183). The importance of consideration of the actual type of PUFA (ω -3, ω -6) or MUFA or some case of individual fatty acids has since developed further (65, 86, 112, 183).

Substitution of SFA by complex carbohydrate also reduces serum TC and LDL-C (66, 67). Additional benefits include reduced energy intake (14, 158), high satiation (degree of fullness leading to meal cessation) (24, 103) and improved bowel function due to the effect of viscous soluble and insoluble dietary fibre (19, 28, 53, 158, 167) and improved glycaemic response, particularly associated with the resistant starch content (53, 69, 71, 158, 167).

Obesity is a risk factor for CVD and also for diabetes, sleep apnoea, some cancers, gastrointestinal disorders and osteoarthritis and relates to all cause mortality (20, 55, 84, 178, 180). Studies have shown that even modest weight reduction ($\leq 10\%$) can significantly improve glycaemic response, insulin sensitivity and lipid profile (59, 82, 174, 180). Research into optimal diets to help combat obesity has suggested reducing dietary total fat, in an effort to reduce energy intake and promote weight loss and increasing protein, complex carbohydrate and dietary fibre intake to facilitate high satiation (14, 21, 67, 142, 181). Recent research into biomarkers for satiation and satiety (interval between cessation of one meal and initiation of the next) has

highlighted the hormones ghrelin and leptin as possible quantitative indicators of the effect of dietary manipulation on appetite and energy intake (31, 137, 158).

Leptin is a protein secreted predominantly by adipocytes and is regulated by insulin-mediated changes in adipocyte triacylglycerols content (56). It acts primarily via the hypothalamus to inhibit stimulation of appetite (31, 137, 187) but also influences thermogenesis (137), the immune system, neuroendocrine function (187), hepatic insulin action, peripheral glucose utilization (56) and ghrelin secretion (76). In states of energy balance, content and timing of meals does not acutely affect leptin concentrations (31) but in a state of fasting, levels drop dramatically, independent of changes to body fat mass (54, 56). Re-feeding or insulin administration quickly restores pre-fasting concentrations (187). Increased concentrations do not elicit the same degree of response as reduced levels, suggesting the physiological role of leptin is to ensure adequate energy intake in times of scarcity rather than as prevention of over-feeding in times of plenty (56, 187). Leptin is more sensitive to dietary carbohydrate than fat, so long term, a reduced energy high-carbohydrate diet may be more effective at maintaining leptin levels and suppressing appetite compared to a reduced energy low-carbohydrate diet (31).

Ghrelin is a polypeptide hormone expressed by endothelial cells primarily in the fundus of the stomach (137). It also acts in the hypothalamus, by stimulating appetite (31, 83, 187). In short-term energy regulation, ghrelin

concentration is strongly associated with appetite and hunger ratings (31). Food entering the stomach suppresses ghrelin in proportion to caloric load (83) but stomach distension does not affect ghrelin concentrations (31, 187). Reduced post-prandial ghrelin concentrations slowly return to pre-meal levels, contributing to initiation of the next meal (31, 83). In long-term energy balance, ghrelin stimulates appetite after weight loss (31). Dietary carbohydrate suppresses ghrelin secretion more effectively and for longer than dietary fat (31), suggesting diets high in dietary fibre and complex carbohydrate should promote higher satiation (via stomach distension) and prolong satiety (via ghrelin suppression) compared to high fat meals, leading to potential weight loss without hunger.

1.2 Legumes and pulses

Legumes are one of the oldest cultivated plant foods (48, 115). There is evidence of their tillage in South East Asia almost 1000 years before the birth of Christ. They grow throughout the Middle East, Africa, the American continent, China and India. There are over 13,000 species of legume; of which approximately 20 are commonly consumed by humans (43, 48, 134, 160). The family is divided into two classes: oil seeds (soybean, peanut, lupin and winged bean) and grain legumes (dry beans, peas and chickpeas) (48, 160). The oil seeds are cultivated primarily for their protein and oil content, the grain legumes as a protein source (48). Legumes grown for human consumption are also known as 'pulses', from the Latin word 'puls', a form of porridge made from dry beans (48, 160).

As is illustrated in Table 2.1, legumes abound in plant protein which is rich in lysine and arginine, but poor in the sulphur containing amino acids (SAA) such as methionine and tryptophan (48, 115, 191). Conversely, cereal grains lack lysine but are rich in SAA. Vegetarian-based cultures traditionally incorporate legumes into their cereal-based diet, thus accessing the full complement of amino acids (43, 48, 134, 184). Legumes are also a good source of complex carbohydrate, dietary fibre (viscous soluble and insoluble), resistant starch (53, 167), unsaturated fatty acids, vitamins, minerals and antioxidants (115, 134, 150, 167). In a report for the United States Department of Agriculture (USDA), legumes, as a food group, were found to contain the greatest amount of total dietary fibre (mostly insoluble) and the least amount of simple sugars (94).

Table 1.1 Selected nutrient content per 100g dry raw weight of soybeans and chickpeas (legumes) compared to brown rice and wheat (cereals).

		Soybeans	Chickpeas	Brown rice	Soft wheat
Water	g	8.54	11.5	12.4	10.4
Energy	MJ	1.74	1.52	1.52	1.42
Protein	g	36.5	19.3	7.5	10.7
Total fat	g	19.9	6.04	2.68	1.99
- saturated	g	2.88	0.63	0.54	0.37
- polyunsaturated	g	11.3	2.69	0.96	0.84
- monounsaturated	g	4.40	1.36	0.97	0.23
Carbohydrate	g	30.2	60.7	76.2	75.4
Dietary fibre	g	9.30	17.4	3.40	12.7
Minerals					
Calcium	mg	277	105	33	34
Iron	mg	15.7	6.24	1.80	5.37
Magnesium	mg	280	115	143	90.0
Phosphorus	mg	704	366	264	402
Potassium	mg	1797	875	268	435
Copper	mg	1.66	0.85	0.28	0.43
Vitamins					
Ascorbic acid	mg	6.00	4.00	0.00	0.00
Riboflavin	mg	0.87	0.21	0.04	0.11
Pantothenic acid	mg	0.79	1.59	1.49	0.85
Folate	mcg	375	557	20.0	41.0

Modified from (172)

1.2.1 Pulses and lipid profile

One of the earliest dietary intervention studies involving pulses was that of Meeker and Kesten in 1940 (87). They demonstrated that in rabbits, animal protein (casein) was potentially more atherogenic than plant protein (soy). A difference in amino acid composition of the two proteins was hypothesised as the responsible feature. Subsequent studies in animals supported the results of Meeker and Kesten, but results of studies in humans have been less convincing (8). In 1995, the effects of dietary soy protein on serum lipid concentrations in humans were investigated via meta-analysis (8). Twenty-nine articles published during the years 1967 to 1994, reporting on 38 studies were evaluated. Analysis indicated that 31 g - 47 g of soy protein per day might significantly reduce the levels of TC, LDL-C and triacylglycerols in hypercholesterolaemic individuals. Sixty to seventy percent of the effect of soy protein was attributed to the effect of soy oestrogens (8). The hypocholesterolaemic effect of soy oestrogens has been supported by a more recent meta-analysis of 23 randomised controlled studies conducted between 1995 and 2002 (190). Included were studies investigating the effect on serum lipid profile of soy protein with isoflavones intact, soy protein depleted of isoflavones and purified isoflavones. Only soy protein with isoflavones intact was associated with significant reductions in serum TC, LDL-C and triacylglycerols and small but significant increase in serum high-density lipoprotein cholesterol (HDL-C). This was postulated as being due to an interaction between the soy protein and associated isoflavones. Degradation of protein and/or isoflavones during the extraction procedure used to isolate them might have been a reason no hypolipidaemic effect was

observed with either depleted soy protein or purified isoflavone extract. As with the previous meta-analysis, the effect of soy was greater in hypercholesterolaemic subjects but also greater in men compared to women and in pre-menopausal compared to post-menopausal women. The latter finding supports the suggested mechanism of action of soy (plus isoflavones) on lipid metabolism through the biological similarity of isoflavones to oestrogen. It was suggested the effect of soy isoflavones on the lipid profile was inconsistent, due to the heterogeneity of results. This last point is supported by a science advisory from the American Heart Association (42), stating soy protein plays only a minor role in lowering LDL-C and its benefit is probably indirect – substitution of animal protein leading to reduced consumption of SFA and dietary cholesterol.

Proposed mechanisms of action of pulses on blood lipid profiles include enhancement of bile acid excretion - resulting in reduced absorption and increased excretion of cholesterol and increased bile synthesis; disruption of the hepatic metabolism of cholesterol and hormonal effects. Enhancement of bile acid excretion has only been successfully demonstrated in some animal models (7, 8, 40, 132). Direct effect on hepatic metabolism of cholesterol has been suggested to occur in one of three ways: increase in HMG-CoA reductase activity; increased removal of LDL-C and very low density lipoprotein cholesterol (VLDL-C) by hepatocytes and human mononuclear cells and/or increase in cholesterol saturation of bile (7, 40, 119, 132, 190). Hormonal effects include possible increases in thyroid hormones, resulting in changes to hepatic metabolism of cholesterol and a decrease of the

insulin:glucagon ratio – an indicator of insulin resistance (8, 132). While many of these mechanisms are attributed to soy protein in particular, other compounds that may have an effect include saponins, phytic acid, trypsin inhibitors, dietary fibre, isoflavones, sterols and stanols (132, 190).

Leguminous protein is rich in arginine (and its precursor L-glutamine), the amino acid substrate for endothelial nitric oxide – an important modulator of vascular tone, haemodynamics and endothelial function. Arginine itself also has physiological effects independent of nitric oxide, including modulation of immune function and inflammatory response, insulin and glucagon secretion and regulation of cardiovascular function (34, 168, 186).

All legumes contain less desirable constituents termed antinutrients or antinutritional factors (36, 48). Until recently a number of this group of compounds were considered detrimental to good nutrition and hence the name. Some, such as lectins (haemagglutinins) and the lathyrus toxin, are toxic to humans, and others such as the oligosaccharides raffinose and stachyose are responsible for the flatulence often associated with consuming pulses (48, 114). Even so, it is the other fermentation products of oligosaccharides - short chain fatty acids such as propionates and acetates, which are thought to play a role in the interruption of hepatic cholesterol synthesis (7, 51).

Protease inhibitors to enzymes such as trypsin and chymotrypsin inhibit the digestion of proteins and α -amylase inhibitors such as tannins and phytic

acid affect the digestion of carbohydrates (48). While pulses are a rich source of vitamins and minerals, the effects of phytates and oxalates reduce the bioavailability of these essential compounds (48, 115, 126). However, recent studies suggest that phytic acid and the 'trypsin and chymotrypsin inhibitor' may have antioxidant effects (114) and the effect of phytic acid on nutrient absorption has only been shown in vitro (43). In addition, the low concentration of inhibitors ingested at usual levels of fibre consumption may result in minimal interference of nutrient absorption (43).

Saponins are a common constituent of pulses. They are poorly absorbed and contribute to the poor absorption of other nutrients (51). They achieve this by forming insoluble complexes with the mixed micelles containing bile salts and cholesterol. Animal studies have suggested that these micelles may contribute to reduced absorption of bile and cholesterol from the intestine and thus contribute to enhanced bile acid excretion (114). Plant sterols and stanols may also contribute to reduced cholesterol absorption from the intestine by replacing it in the mixed micelles and being transferred into the enterocytes instead of cholesterol (32).

Many studies have suggested that soluble fibre has a greater effect on lowering human serum lipid levels than insoluble fibre. One literature review investigating the hypocholesterolaemic effect of dietary fibre from a number of food sources (50) suggested that soluble fibre lowered serum TC and LDL-C but had no effect on serum HDL-C or triacylglycerols. The food sources investigated included pulses (dried beans, peas, chickpeas, lentils); cereal

grains and brans; fruit pectin, guar gum (from a leguminous seed), psyllium and other sources of dietary fibre. Discussing factors to consider when evaluating fibre studies, the authors noted the influence of the type and amount of fibre in the test and control diets, the effect of the fibre supplements on the fat and carbohydrate content of the diets and the baseline lipid levels of the participants.

A dietary intervention trial (153) researching the effect of soybean polysaccharide reported a significant reduction in plasma TC in 31 free-living volunteers aged between 25 and 67 years with mildly elevated TC. The participants consumed cookies and croutons containing either 25g of soy polysaccharide per day or starch placebo during two consecutive, randomised crossover dietary periods. There was an order effect however, with the polysaccharide/placebo group showing an 11% decrease in plasma TC (28mg/dl) compared to a 5% decrease for the placebo/polysaccharide group (11 mg/dl). An ordered crossover study (95) reported that adding 25 g/day of soy fibre to an already low-fat low-cholesterol diet resulted in a further reduction in serum TC of 13 mg/dl and in LDL-C of 12 mg/dl ($p < 0.05$) after that elicited by the 12-week low-fat low-cholesterol diet alone. These results suggest a hypocholesterolaemic effect of dietary fibre independent of baseline lipid level but the study only comprised 11 individuals.

Another intervention study observed the effect of pulses on serum lipids and lipoproteins when added to an already low-fat diet (75). In this case, significant decreases in serum TC (7%) and triacylglycerols (25%) were

reported after 16 weeks consumption of a low fat diet in which 25% of total energy per day from carbohydrate was supplied by pulses. Serum LDL and HDL-C concentrations were unaffected. While the fat, protein and carbohydrate content of the altered diet was held constant, dietary fibre and starch content were increased by 66% and 12% respectively. There was also a significant reduction in dietary cholesterol (32%), perhaps due to the higher leguminous protein content replacing some animal protein in the diet. Even so the result must be treated with caution because the study population was very small and of only one gender – male. After the trial, five of the seven men chose to continue including legumes in their diet; a move interpreted by the authors to indicate easy acceptability of legumes into a Western diet; however, no follow-up was reported, so there was no indication of the permanence of the dietary change.

A controlled, parallel, dietary intervention study compared the effect of two test diets - oat bran and dry bean products (pinto and navy beans) to a control diet on the blood lipids of 20 hypercholesterolaemic men (34 to 66 years old) while they were in-patients on a metabolic ward (10). The test diets were of 21 days duration, following on from a seven-day control diet. Both test diets were isocaloric with their respective control diets except for dietary fibre (approx. 60% more total and 65% more soluble fibre). Results demonstrated a 19% reduction in serum TC following both test diets and a 23% reduction in LDL-C following the oat-bran diet and a 24% reduction following the bean diet compared to their control diets. Although serum HDL-C levels were reduced after both test diets (oat bran: 6% less; bean: 13%

less), the HDL:LDL ratio increased by 17% and 22% respectively. There was an average weight reduction of 1.0 kg - 1.3 kg during both the test diets, which was statistically significant compared to their respective control diets but not between the two test diets. Energy consumption during the bean diet was slightly lower than the control but not significantly different. The bean group consumed slightly more PUFA and slightly less SFA during the test phase but the difference was not statistically significant from their control diet or the oat-bran group. These small differences in energy and nutrient intake may have affected serum cholesterol concentrations during the bean diet to a minor degree but no such differences in nutrient intake were apparent during the oat-bran diet and there was no significant correlation between change in serum TC and weight reduction. After the trial, participants were encouraged to continue on similar diets high in fibre from oat and bean products. Follow up of 10 participants after 24 weeks demonstrated continuing reductions in TC and LDL-C. Follow up of four participants after 99 weeks demonstrated an increase in HDL-C. These continued changes may have been due to adherence to a high fibre diet but other lifestyle changes that often accompany dietary change (eg. increased physical activity and weight loss) may have also contributed to the continuing changes in lipid profile. Results from such a small group would be difficult to interpret.

Some animal studies examining the effects of legumes have also reported reductions in LDL-C concentrations. One intervention study recorded the effects of hypocholesterolaemic diets supplemented with four different legumes (baked beans, peas, lentils, butter beans) on the lipid profile of pigs

(81). After six weeks, results indicated all four diets significantly inhibited hypercholesterolaemia by suppression of LDL-C and VLDL-C production. Another study compared the effects of diets supplemented with either cereal or legume fibre, on the lipid profiles of hypercholesterolaemic rats (166). The legume diets produced significantly lower plasma and tissue LDL-C and VLDL-C levels compared to the cereal diets. The physical and chemical properties of cereal and legume fibres were also analysed. Results indicated soy and chickpea fibre to have greater bile binding capacity than fibre from wheat or maize. Consequently, it was suggested that the hypocholesterolaemic effect of legume fibre was associated with its greater bile binding capacity. It was argued that this property was responsible for reduced reabsorption of bile in the colon, resulting in increased bile synthesis and reduced cholesterol synthesis. However, while these studies may provide proof of concept and help explain possible mechanisms for the hypocholesterolaemic effect of legume fibre, the results cannot be directly translated to the human condition.

Comparison of fibre from oat-bran and beans was the subject of an intervention study investigating their effects on human serum lipids and lipoproteins in combination with a low-fat diet (99). The study population numbered 39 free-living, hypercholesterolaemic men and women, aged 28 to 66 years. The intervention diets were administered in a random crossover design. They differed in the source of dietary fibre (beans or oat-bran) and the amount of β -D-glucan content (high fibre oat-bran or low fibre oat-bran). In contrast to the study of Anderson et al (1984) (10), results of this study

indicated a small but significant increase in plasma HDL-C after all three test diets but no significant effect on LDL-C or TC. It was suggested that other studies might not have detected a rise in HDL-C due to an inadvertent reduction in saturated fat intake or changes to the P:S ratio. These changes may have been responsible for reducing serum HDL-C levels, thereby masking any rise due to other dietary factors (99).

Another intervention study found no change in the serum lipid levels of nine healthy human males aged 19 to 28 years, subjected to three weeks of a typically Western diet supplemented with green lentils (163). The authors proposed that the lack of effect on serum lipids might be due to the normal baseline lipid profiles of their participants (7, 22, 50), or the fibre composition of lentils, which is primarily insoluble fibre. Then again, the length of the intervention diets may not have been long enough to allow a significant change in serum lipids to take place (4, 145, 146) and the study may not have been powerful enough to detect a difference in serum lipid levels due to the small number of subjects.

While animal and human dietary intervention studies have indicated a potentially beneficial effect of legumes on serum lipids, observational studies are inconclusive regarding an association between legumes and reduction in CHD mortality (90). An epidemiological study in support of an inverse relationship between legume consumption and CHD, is the follow up of the First National Health and Nutrition Examination Survey (NHANES 1), the Epidemiologic Follow up Study (NHEFS) (17). The study consisted of results

from 9632 men and women, aged between 25 and 74 years at baseline, collected over a period of 19 years. Legumes ('dry beans and peas like pinto beans, red beans, black-eye peas, peanuts and peanut butter') were one of 13 food categories included in a three-month recall, food frequency questionnaire, conducted at baseline. At the completion of the study, the investigators reported a strong inverse association between dietary intake of legumes and risk of CHD, independent of the other established CHD risk factors such as smoking and saturated fat intake. It must be noted, however, that information on portion size was not collected in the questionnaire but estimated by one 24-hour dietary recall. Depending on the time of year, a three-month dietary recall may be subject to seasonal variation in diet. Thus, the accuracy of legume intake might be questioned. In addition, legume consumption (and overall dietary intake) was estimated at baseline only – change in dietary habit was not assessed during the 19-year follow-up. While 'in depth interviews' were performed during follow-up, no details are given as to assessment of lifestyle changes that may have confounded the outcomes.

A meta-analysis of clinical trials investigating the effects of 'non-soya pulses' concluded that regular consumption of pulses may protect against risk of CVD (9). Eleven clinical trials conducted over the previous 20 years were identified and although they varied greatly in design and setting, the authors included all of them 'to avoid biases'. Meta-analysis revealed a decrease in TC of 7% and LDL-C of 6% associated with the ingestion of pulses but no effect on HDL-C. No mention is made of the required amount of pulses that would bring about this level of decrease in serum lipids. It was postulated that

the hypocholesterolaemic effect of pulses was due to a combination of their nutrient components (eg soluble fibre, vegetable protein, oligosaccharides, isoflavones, phospholipids and fatty acids), rather than the individual components themselves. It was suggested that an increased consumption of pulses may have a beneficial effect on other CVD risk factors such as obesity, diabetes and hypertension in addition to hypercholesterolaemia.

1.2.2 Pulses and glycaemic control

Following on from studies into the healthful effects of dietary fibre by Burkitt and Trowell, researchers became interested in the effect of dietary fibre on glycaemic response (100, 155). It had been noted that different sources of carbohydrate had differing effects on post-prandial blood glucose levels, depending on the physical form of the food, the nature of the carbohydrate, the amount of dietary fibre and the presence of other macronutrients (74, 97, 165). The glycaemic index (GI) was devised to predict the effect of an equal quantity of carbohydrate in a food on post-prandial glycaemic response compared to a control food – initially a combination of cottage cheese and white bread (73). Foods categorised as low GI produced a lower, flatter glycaemic response, reflecting the slower, steadier rate of glucose absorption over a longer period, from food more slowly digested, allowing the body easier transition from the ‘post-prandial to post-absorptive state’ (73, 97). The glycaemic load (GL) better reflects the ‘glycaemic impact’ of a food portion (15) by taking into account the amount of available carbohydrate as well as its GI. Thus, a carrot and a potato both have a high GI but the carrot has a low GL because there is less carbohydrate per standard serve (97). Diets

containing large amounts of high GI and GL foods contribute to higher fasting and post-prandial blood glucose and insulin concentrations in both diabetic and non-diabetic individuals, possibly resulting in greater risk of developing diabetes, heart disease and cancer (165). In addition, the post-prandial response to a high GI meal often results in insulin-induced hypoglycaemia with ensuing desire for additional food intake, leading to unnecessary weight gain (97).

In a study comparing the effect of fibre from different sources, soy hulls were included as one of three processed fibres tested (100). After four weeks of daily supplementation of their habitual ad libitum diet with either 26 g or 52 g soy hull fibre, incorporated into bread, 18 free-living adults diagnosed with non-insulin dependent diabetes mellitus (NIDDM) recorded very slight improvements in glycaemic control compared to the white-bread four-week control phase. Improvements occurred in fasting plasma glucose, glucose score and post-prandial glucose tolerance testing after a formula meal. The results suggested a 'lingering', longer-term effect of soy hull fibre on glycaemic response as well as the post-prandial 'acute effect'. However, results were inconsistent between individuals and the groups and confounded by variation in bodyweight.

In one of the earliest studies to report on the post-prandial glycaemic effect of legumes as a whole food, eight different varieties of dried legume were compared to 27 other high carbohydrate foods, including processed legumes, dried and processed cereals, cereal products and starchy vegetables (74). As a class, legumes produced approximately half the post-prandial glycaemic

response of any of the other food groups. This was attributed to their high dietary fibre content in combination with starch in a form more resistant to digestion. A study examining the effect of legumes common to Indian diets on glycaemic response determined that the GI of the foods tested – kidney beans, Mung beans, chickpeas, rice and wheat was directly proportional to the amount of viscous dietary fibre they contained (38). The hypothesis was that viscous polysaccharide increased the thickness of the ‘unstirred’ layer surrounding intestinal villi, thereby reducing the rate of glucose absorption. More recent studies indicate that glucose absorption is affected by the rate of gastric emptying which is in turn affected by the energy density of the diet (188). Viscous polysaccharides cause delayed gastric emptying and slower intestinal transit, resulting in a reduced rate of glucose absorption (19).

The effect of legumes as a whole food was also investigated in a randomised cross-over study using six-week interventions, involving 27 diabetic individuals (18 NIDDM and nine insulin dependent diabetes mellitus - IDDM) (155). The effect of a high leguminous diet (HL) was compared to a ‘traditional diabetic diet’ (low carbohydrate, LC), on post-prandial and longer term glycaemic response and the serum lipid profile. The HL diet produced significantly lower basal, preprandial and two-hour postprandial glycaemic responses compared to the LC diet but insulin response, though lower during the HL diet was not significantly different. Mean body weight was 0.9 kg lower after the HL diet, even though the diets were devised to maintain weight but there was no correlation between degree of weight loss and glycaemic response. Mean plasma TC was significantly lower after the HL

diet compared to the LC but plasma LDL- and HDL- results varied between the NIDDM and IDDM groups. Comparison of the intervention diets shows them both contributing 21% of total energy (%E) per day from protein. The HL diet contributed 61 %E from carbohydrate and 18 %E from total fat. The P:S ratio was 1:1 and the dietary fibre intake was 97 g/day. In contrast, the LC diet contributed 40 %E from carbohydrate and 39 %E from total fat. The P:S ratio was 1:3 and the dietary fibre intake 15 g/day. The glycaemic effect of the HL diet was attributed to the 'large quantities of leguminous fibre' but no distinction was made between differing forms of carbohydrate. The hypocholesterolaemic effect of the HL diet may have been due in part to substitution of carbohydrate for dietary fat, substitution of PUFA for SFA or the effect of high dietary fibre intake. It would be hard to convince diabetics to adhere to the HL diet for any length of time, due to the large amount of legumes they would be required to consume.

A later study investigating the effect of a high-carbohydrate, high-fibre diet (HCHF), utilizing 'whole-grain or bran cereals, dried beans, vegetables and fruits' compared to a low-fibre, low-carbohydrate diet (LCLF) on glycaemic response and lipid profile, reported different results (11). The randomised crossover study involved interventions of four weeks duration separated by a six-week washout. The HCHF diet provided 10 %E from total fat (P:S ratio 2:1), 20 %E from protein, 70 %E from carbohydrate (25% simple, 75% complex) and 35 g fibre/1000 Cal – 56g/day to 89 g/day (approx. 1/3 soluble fibre). The isoenergetic LCLF diet provided 41 %E from total fat (P:S ratio 1:2), 20 %E from protein, 39 %E from carbohydrate (50% simple, 50%

complex) and 5g fibre/1000 Cal – 9 g/day to 13g/day (approx 1/3 soluble fibre). Significantly lower mean basal insulin requirements were produced during the HCHF diet compared to the LCLF but there were no significant differences in mean fasting or post-prandial plasma glucose concentrations. Mean baseline body weight was not significantly different between the diets and there was no significant change in body weight during the two intervention periods. As a measure of peripheral tissue insulin sensitivity, the carbohydrate: insulin ratio (C:I) was also determined in this study. This ratio compares the amount of carbohydrate consumed to the amount of insulin delivered during a set period and it was found to be significantly greater during the HCHF diet, compared to the LCLF. This increase in peripheral tissue insulin sensitivity was thought to be responsible for reducing the amount of insulin required during HCHF diets resulting in improved long-term glycaemic response. Other modes of action that may have contributed to lower basal insulin response included increased chewing time for the higher-fibre low-energy dense foods – making eating a longer, slower process; slowed gastric emptying due to increased amounts of soluble dietary fibre; slower absorption of glucose from the small intestine due to resistant starch content; decreased secretion of GIT hormones; increased production and absorption of short chain fatty acids (from colonic fermentation of insoluble dietary fibre and resistant starch) and their effects on glycolytic enzymes in the liver and skeletal muscle (11, 46). As with a number of previous studies, the population sample in this study was quite small – ten participants and 90% male.

The effect of HCHF diets on peripheral tissue insulin sensitivity was investigated in a small group of healthy young and old adults but again the groups were very small (n=12: six younger, six older) (46). In addition, the six younger participants were all male but three of the older participants were female. A euglycaemic insulin clamp and (6, 6-²H₂) glucose measured insulin-mediated glucose disposal and hepatic glucose production before and after a three to four-week isoenergetic HCHF dietary intervention. In the 12 healthy adults tested – especially in the six young men, the HCHF diet significantly improved insulin-mediated glucose disposal and thus peripheral tissue insulin sensitivity, compared to the habitual *ad libitum* diet of the participants with no change in hepatic glucose production. Fasting plasma glucose, insulin and serum TC were all significantly reduced following the HCHF intervention – serum TC more so again in the six younger men compared to the six older adults but serum triacylglycerol concentrations remained unchanged. The hypothesis was that the metabolic effects of HCHF diets on glycaemic response could be due to effects of dietary fibre on either intestinal absorption of glucose, hepatic glucose output or peripheral tissue insulin sensitivity. But in this study there were large differences in total fat and fatty acid content as well as vast differences in dietary fibre consumption during the HCHF intervention compared to the control (the participants' habitual *ad libitum* diet). During the HCHF diet, mean fibre consumption ranged from 70-100 g/day compared to 10-18 g/day normally; total fat consumption was 66% less during the HCHF and the P:S ratio altered from 1:2 (habitual diet) to 1.5 :1 (HCHF diet) for the younger group and from 1:2 to 1:1 for the older group.

In more recent articles reviewing the health-benefits of legumes, they are described as rich in 'slow release carbohydrate' (167), dietary fibre and protein (53, 167). The resistance of legume starch to digestion compared to cereal starch is explained as being due to the higher percentage content of amylose, the greater content of retrograded starch in processed legumes and the increased protein content (starch-protein interactions). In addition, the presence of amylase inhibitors helps neutralise the digestive enzymes; phytates form large insoluble nutrient complexes and dietary fibre physically binds the starch, preventing or slowing dispersion and reducing exposure to digestive processes. Diets high in legumes and low in SFA have been suggested to have greatest long-term benefit to individuals suffering from diabetes or hypercholesterolaemia (126, 139) although the mechanism is not clearly defined (121, 126). Such individuals are commonly also obese (55) and weight reduction is known to improve lipid profile and insulin sensitivity (59, 82, 173, 174, 185). Some authors believe short term glycaemic change is associated with macronutrient change and energy restriction, while longer term change in insulin sensitivity is associated with weight loss and altered abdominal adiposity (58, 102). Others believe dietary macronutrient content, especially SFA and dietary protein are important determinants of peripheral tissue membrane permeability to insulin and glucose (91, 164, 177). One author suggested the effect of carbohydrate not digested in the small intestine is responsible for beneficial consequences to cholesterol and glucose metabolism as well as on colonic health and function (126).

1.2.3 Pulses and bowel function

Poor or slow starch digestibility leads to lower, slower glucose absorption in the small intestine with greater amounts of starch reaching the large bowel where, along with soluble dietary fibre, it is completely fermented, with partial fermentation of insoluble fibre (19, 28, 53, 167). Consumption of a high protein diet, may lead to undigested protein reaching the large bowel.

Fermentation of protein leads to formation of toxic, potentially carcinogenic compounds such as phenol, cresol, indoles, ammonia and amines (98).

Increased amounts of fully and partially fermentable carbohydrate result in increased growth of colonic microflora, dilution of toxic metabolites, increased faecal bulk, shortened bowel transit time and softer, bulkier stools (19, 28, 53, 167). The production of short chain fatty acids is a by-product of carbohydrate fermentation – in particular acetate, butyrate and propionate.

Fermentation of soy fibre leads to increased synthesis of propionate and butyrate compared to other highly fermentable fibres (53). Absorbed propionate is hypothesised to interfere with hepatic cholesterol (19, 28, 53, 167). and glucose metabolism (126), to enhance colonic muscular contractions and increase colonic blood flow (167). Butyrate is the nutrient of choice for normal colonocytes, reducing risk of malignant transformation and colon cancer.

One study investigating the effect of leguminous dietary fibre on human colonic function overall, examined how supplementing a 'Western' diet (control diet) with 130 g/day of green lentils (lentil kernel fibre - LKF diet) for

three weeks affected colonic function (163). Again the population group was very small and only one gender– nine healthy adult men. The diets were isoenergetic and similar in macronutrient content, apart from dietary fibre (non-starch polysaccharide – NSP) consumption, which was 11.8 g/day more during the LKF diet due to the lentils. It was found that during the LKF diet faecal weight increased by 45% and faecal nitrogen excretion increased by 30% compared to during the control diet. This suggested that the increase in faecal weight was due to increased bacterial mass from fermentation of NSP rather than increased water holding capacity exhibited by fermentation-resistant dietary fibre, such as wheat bran. The increase in faecal nitrogen was greater than expected from the 75% of lentil NSP fermented because other forms of carbohydrate contained in lentils such as oligosaccharides and starch ‘resistant to small intestine digestion’ may have contributed. One earlier study using navy and pinto beans, found that leguminous fibre did not contribute to increased faecal weight while another study using soy polysaccharide did. Even though faecal weight increased substantially during the LKF diet, mean bowel transit time (BTT) was not significantly different. This might have been because the participants, who were healthy young men, already displayed relatively fast BTT’s on their usual diets so faster times would have been hard to detect.

A more recent study determined the effect of adding pea-hull fibre to the usual diet of elderly institutionalised participants (29). The six-week intervention involved the substitution of wheat flour for 4 g/day of finely ground pea-hull fibre in the usual meals of 114 elderly men and women. This

increased the mean daily dietary fibre intake from 16 g to 20 g – if all foodstuffs were eaten. The frequency of administration of laxatives and enemas and the number of bowel movements experienced by the participants during a four-week baseline period was compared with the middle four-weeks of pea-hull treatment. The pea-hull fibre used had a total dietary fibre content of 78% - 71% insoluble and 7% soluble fibre. There was a significant increase in bowel movements per month for all residents during the pea-hull treatment compared to baseline. A small group of residents with bowel movements of less than ten per month during baseline, considered at risk of constipation, also showed a significant increase in bowel movements during the pea-hull treatment. Frequency of administration of fruit/prune puree was reduced but not administration of laxatives or enemas during the pea-hull treatment but this may have been due to overuse of the procedure rather than no change in need of administration. Acceptance of the pea-hull fibre and the bowel health benefits were such that the institution continued to incorporate pea-hull fibre in the menus offered to their residents after completion of the study.

1.3 Chickpeas

Chickpeas are most commonly associated with the cuisine of the Mediterranean and Asia - especially India (48, 160). The number of synonyms: Bengal gram, boot, kabli chana, chana chola, chole, kaala, gram, hommes, pois chiche, garbanzo bean, barbarzo bean, is consistent with a long history of use throughout these regions. The scientific name, *Cicer arietinum*, is derived from the Roman name for chickpeas. The Roman

family, Cicero, took their name from the chickpea, and arietinum is the Roman word for ram. Apparently, the shape of the chickpea looks like a ram's head - complete with curling horns (48, 160).

The two cultivars, Desi and Kabuli, are grouped according to seed colour and geographic distribution. Desi are common to India. They are smaller and range in colour from fawn to brown, yellow, orange, black or green. They are eaten either whole, as dhal (puree) or dhal flour. The Kabuli cultivar is more common to the Mediterranean. The pea is larger, white in colour and usually eaten whole. Nutritionally, Kabuli chickpeas are very slightly higher in protein content and fat, however Desi chickpeas provide more than three times the dietary fibre (129). Chickpeas are the second most cultivated pulse worldwide and are third largest in terms of amount of pulse produced worldwide (129, 157, 191). They are a very important staple food for developing countries because they provide a cheaper form of protein than expensive animal sources. In addition, they are easy to grow - even in harsh arid environments, and are acceptable to the mostly vegetarian and semi-vegetarian cultures that inhabit the Indian and Mediterranean regions (129).

Per 100 g, dried chickpeas contain 19.3 g of protein, which compares favourably with wheat at 10.7 g. Chickpea protein digestibility (75-84%) is the highest among the dry edible legumes, perhaps due to chickpeas having the lowest concentration of trypsin inhibitors (18, 68, 122). A big advantage of plant-sourced protein over animal proteins is the accompanying nutritional extras: dietary fibre, complex carbohydrates and no cholesterol. The

carbohydrate of both chickpeas and wheat is composed mainly of starch with a small residue of sugars and oligosaccharides (43). Although chickpeas contain less carbohydrate than wheat, the starch they do contain has a higher amylose content (46%), which renders it more resistant to digestion (126, 135). The seed coat of chickpeas is removed as part of the food preparation process (dehulling). Thus the dietary fibre content, at 17 g/100 g, is not as high as other pulses consumed with their seed coat intact (51). Even so, compared to cereal grains chickpeas are a very good source of dietary fibre, both soluble and insoluble plus resistant starch. In contrast to most other pulses and cereals, chickpeas have a relatively high fat content at 6 g/100 g. This makes them an important energy source for vegans and those without regular access to meat and dairy products. The fat is mostly ω -6 PUFA, with some MUFA and less than 1% SFA (172).

Chickpeas are a rich source of vitamins, minerals and phytoestrogens. They contain folate, thiamine, riboflavin, niacin, pantothenic acid, vitamins C, A and E (51). Chickpeas have a higher content of calcium and phosphorus than other pulses and are a good source of iron and zinc (114, 129). They also contain magnesium, copper, manganese and selenium (51). Chickpeas are abundant in the isoflavones formononetin and biochanin A, phytoestrogens common to many pulses (108, 118, 149, 151, 154). Chickpeas are relatively free of antinutrients, such as lectins, but do contain small amounts of saponins, oligosaccharides, some tannins and phytate (51, 129, 134).

1.3.1 Chickpeas and serum cholesterol

Relatively little clinical or nutritional notice has been taken of chickpeas by the general scientific community - even though they are an important staple food source in third world countries (191). Most of the earlier investigations were conducted using laboratory animals, and mainly by Indian investigators. In the 1960's, a series of studies using rats and rabbits resulted in evidence that suggested inclusion of chickpeas in the diet would have a significant effect against high serum and tissue cholesterol concentrations. In one experiment, groups of rats were fed hypercholesterolaemia-inducing diets (HID's) supplemented with whole chickpeas, chickpea lipid extract or the defatted chickpea residue for a period of six weeks (105). Results of samples taken at the completion of the diets suggested that all three forms of chickpea supplement significantly reduced raised serum cholesterol in rats. As part of the same study, another group of rats were fed HID's for six weeks and then HID's plus one of the three forms of chickpea supplement. Again, all three forms of chickpea supplementation prevented a rise in cholesterol but no data is given of cholesterol levels at the completion of the HID phase, before the supplemented phases began. Furthermore, to keep the diets isocaloric, the content of the HID's were changed, depending on which chickpea supplement was added. The main differences were in the quantity of sucrose in the control diet (60%) compared to the supplemented diets (0%, 5% or 57%) and the presence of casein in the control and lipid extract diet (15%) but not in the whole gram or defatted gram diets. These differences could possibly have contributed to the results obtained.

The author attributed the hypocholesterolaemic action of the chickpea lipid extract to its rich content of linoleic and oleic acid and the hypocholesterolaemic action of defatted chickpea residue to the constituent proteins. In a follow-up study designed to identify the active proteins, rats were fed HID's supplemented with chickpea protein portions fractionated by solubility in either water, saline or alkaline solutions (105). Only the saline soluble portion was reported to significantly lower serum cholesterol. This portion contained the highest concentration of arginine, a protein known to exhibit hypocholesterolaemic properties (8, 105).

In 1973 a group of investigators repeated the study of Mathur but they could not duplicate the results (124). They used rabbits rather than rats as the experimental animal, so perhaps a difference in species lipid metabolism may have contributed to a difference in results. Again, the base diet (HID) included both sucrose (60%) and casein (15%) and the sucrose was reduced in the chickpea-supplemented diet (19%). They reported a marked increase in serum lipids in both groups of rabbits and the presence of grade II atheromatous lesions. They reasoned that 'the excess of sucrose' might have been responsible for the atheromatous lesions. No mention was made of the possible atherogenic effect of casein.

In an experiment of their own, using a different base diet, Nityanand and Kapoor found no change to blood lipid levels or atherosclerosis formation in rabbits (124). The rabbits were fed a stock diet supplemented with chickpea flour and cholesterol, compared to another group fed a stock diet plus

cholesterol. The stock diet contained wheat flour (55%) and chickpea flour (20%) compared to the chickpea-supplemented diet, which contained 75% chickpea flour. Perhaps the presence of chickpea flour, even at only 20%, affected the results obtained using the stock diet. In addition, Nityanand and Kapoor did not include casein or sucrose in their base diets and they used a different form of chickpea to Mathur. These differences may also have affected their results.

Following on from work involving animals, Mathur, Khan and Sharma, conducted a dietary intervention study in humans (106). The study involved 30 healthy middle-aged men (15 to 50 years old) divided into two groups. One group of 20, followed a diet supplemented with 156 g of butterfat for ten weeks (high-fat diet), then a high-fat-plus-chickpea diet for 55 weeks. The control group of ten followed a 'routine hospital diet' containing 35 g of fat for the whole 65 weeks. The results showed a significant decrease in serum cholesterol in 16 out of 20 subjects after the high fat plus chickpea diet. No mention is made of weight gain or loss, no mention is made of usual energy intake or expenditure and no mention is made of results of the control group. The control and test groups were composed of different individuals; differed in number of participants, and possibly in mean age and weight. No mention is made regarding the study setting e.g. free-living, metabolic ward or hospital ward, thus there is no indication of the degree of compliance or dietary control. The high-fat and high-fat-plus-chickpea diets were isocaloric in total energy consumed but differed in composition of carbohydrate and protein. While the fat content of the high-fat and control diets was stated, no

mention is made of the actual chickpea content of the high-fat-plus-chickpea diet or the amount of 'wheat flour and other cereals' the chickpeas replaced. Four subjects did not show a reduction in serum cholesterol and it is not evident whether their results are included in the mean values published, as no indication of the number of results used to calculate the means is given.

One of the first studies to consider the hypocholesterolaemic potential of different dietary fibre from pulses compared the effects in rats, of fibre derived from five pulses commonly consumed in India: Green gram, Black gram, Bengal gram (chickpeas), peas and lentils (159). Six groups of rats were fed hypercholesterolaemic diets supplemented with crude fibre isolates of each of the five pulses or cellulose (control), for four weeks. Results indicated a significant lowering of cholesterol by all five pulses compared to the control, however there was no significant difference detected in the plasma cholesterol-lowering effect between the pulses. As part of the same study, the hypocholesterolaemic effect of fibre as part of the whole pulse was compared to the corresponding crude fibre extract. The hypocholesterolaemic effect per gram of fibre was significantly different between the whole pulses but not between the crude fibre extracts, suggesting an additional effect of some other constituent in the whole pulses.

More recently, whole-germinated chickpeas were included in a human dietary intervention study (49). The study measured the potential hypocholesterolaemic effects of two plant alternatives (chickpeas and garlic) to synthetic lipid-lowering drugs. Thirty normal, healthy participants (19 – 21

years old) were divided into three equal groups matched for age, weight, sex and socioeconomic status, resulting in only ten individuals in each group. The two test groups were placed on a controlled diet plus a daily supplement of either 100 g of whole-germinated chickpeas or 15 g of garlic, for eight weeks. The third, control group consumed the same controlled diet as the test groups supplemented with guggulipid at a dose equivalent to 25 mg of guggulsterone. Guggulipid is an extract of the resin of the guggul tree (*Commiphora mukul*) and has well documented hypolipidaemic properties attributable to a steroid-type compound, guggulsterone (171). At the completion of the diets, the chickpea-supplemented group recorded a 17% reduction in serum TC, the garlic-supplemented group recorded a 13% reduction and the guggulipid reference group recorded a 32% reduction. The macronutrient content and energy intake of the 'controlled diet' was not elucidated, nor compared to the usual diet of the volunteers. The setting of the study was not mentioned, however, reference to 'healthy normal volunteers' suggests a free-living environment. The initial serum TC level of the participants determined the three groups. There was no reason given for this arrangement. The group with the highest level (mean 212 mg/dl \pm sd 6.23 mg/dl) was assigned to the guggulipid-supplemented diet; the group with the next highest concentration (157 mg/dl \pm 4.94 mg/dl) was assigned to the chickpea-supplemented diet; the group with the lowest mean initial serum total cholesterol (148 mg/dl \pm 8.81 mg/dl) was assigned the garlic-supplemented diet. It has been suggested that the higher the initial blood lipid levels of participants, the greater the chance of detecting any change due to dietary intervention (5, 48, 138, 175). Thus, the difference in mean initial total

cholesterols in the study of Ghorai et al (49) may have influenced the degree of change obtained at the conclusion of the three treatments. Coupled with the small number of participants in a case-control setting and the lack of information regarding the intervention diets and the study design, the results from such a poor study are difficult to interpret.

The hypocholesterolaemic potential of chickpeas was not widely acknowledged by the Western scientific community until the mid 1990's. The reason for this may have been the preoccupation of the Western scientific community with three main areas: S-amino acid deficiency of legume proteins, the presence of enzyme inhibitors and the presence of lectins (36). This attitude is illustrated by a paper published in 1995 which claims the study, 'of a previously uninvestigated legume', to be the first to demonstrate 'that chickpea consumption may have a corrective effect in some alterations of the lipid profile' (192). The authors compared the effect of a casein diet and a chickpea diet on hypercholesterolaemic rats. There was a certain degree of emphasis placed on the undesirable qualities associated with legume consumption throughout the paper, even though the outcome of the study was very positive regarding the status of chickpeas as a hypocholesterolaemic agent. Significant lowering of TC, LDL-C, VLDL-C and triacylglycerols were reported. This finding was repeated four years later in a study, also on rats, using heated chickpeas (191). The chickpeas were autoclaved before inclusion in the test diet, in the belief that 'some anti-nutritive factors may be reduced or eliminated by the processing method'. The authors reported that no growth impairment was noted in rats fed the

chickpea-supplemented diet; an outcome that had been previously associated with legume consumption (134, 191). Concluding their report, Zulet et al (191) suggested that the inclusion of chickpeas in the diets of people with hyperlipidaemia might be beneficial in correcting their lipid profile; however, more investigation was required.

1.3.2 Chickpeas, glycaemic response

Legumes as a group have been shown to exhibit the lowest postprandial blood glucose response of several carbohydrate-containing foods in diabetic individuals (74). In a later study, calculation of GI of five legumes and seven other carbohydrate-containing foods consumed by diabetic participants, revealed that red lentils, with a GI of 44 ± 7 (mean \pm SEM) and chickpeas (GI: 47 ± 9) displayed the lowest GI, compared to a mixture of white bread and cottage cheese – added to account for the protein content of legumes (73). A study investigating the glycaemic index of commonly eaten Indian foodstuffs found that adding chickpeas to milled rice produced the only significant reduction in GI, from 74 ± 8 (mean \pm SEM) to 54 ± 1 (101). Other combinations tested included rice mixed with whole green peas, whole green gram, green gram dhal (dehusked and split) or red gram (pigeon pea) dhal. The nutrient content of the rice-chickpea mix differed from the other combinations mainly in the amount of total fat contained per serve – 1.5 g compared to 0.5 g, most of which would have been PUFA – hypothesised to be involved in increasing insulin sensitivity (66, 183).

A more recent article reported on investigations to determine whether ingestion of chickpeas altered postprandial and/or long-term glycaemic response and insulin sensitivity compared to an isoenergetic wheat-based diet in non-diabetic individuals (121). The postprandial study involved 19 healthy, middle aged men and women, each consuming three standardised meals on three separate occasions. The meals differed only in source of available carbohydrate, from white bread (control), wheat or chickpea. It was found that plasma glucose concentrations were significantly less 30 and 60 minutes after ingesting the chickpea meal compared to after the wheat or control meals. Furthermore, plasma insulin concentrations and a calculated measure of basal insulin resistance (Homeostasis Model Assessment of Insulin Resistance – HOMA-IR), measured 120 minutes post prandially, were both significantly lower after the chickpea meal than after the wheat or control meals. These results infer that legume (specifically chickpea) consumption contributes to improved post-prandial glycaemic response and insulin sensitivity in healthy participants as well as in diabetic individuals. The longer term, randomised crossover study involved another 19 middle aged male and female subjects completing two periods of controlled dietary intervention, a chickpea-supplemented diet and a wheat-based diet, each of six weeks duration. Dietary fibre intake was significantly greater during the chickpea intervention compared to the wheat; otherwise macronutrient intake during the two interventions was not significantly different. Plasma glucose and insulin concentrations and HOMA-IR scores either fasting or two hours after a glucose loading, were not significantly different following the two interventions. These results suggest that there was no change to long-term

glycaemic response or insulin sensitivity due to consumption of the chickpea-supplemented intervention compared to the wheat. The authors cited other literature that supported this finding although none of the sources could give an explanation as to why short-term improvements did not translate to the longer term. However, body weight remained consistent throughout the study and SFA consumption was unchanged.

Examination of the literature reveals a gradual development of interest in the role of legumes and pulses in a healthy diet and the perceived 'anti-nutritive' consequences of pulses are being countered. Animal models have provided proof of the hypocholesterolaemic action of legumes and their ability to improve glycaemic control but the results haven't translated so successfully to human studies. Chickpeas are a relatively novel addition to some Western cuisines and currently there are very few studies in the literature examining the effects of chickpeas on human health, especially with regard to CVD risk factors such as hypercholesterolaemia, diabetes and obesity. Completed studies have suggested beneficial effects but results have not been consistent for a number of reasons. A difference in location of the studies provides different population study groups that may reflect different responses to intervention diets. The type of chickpea used in the Indian studies (Desi) contains more fibre and less protein than the chickpeas used in the Western studies (Kabuli) and the small number of participants in the small number of human studies prevents accumulation of a decent amount of data for meta-analysis. Thus additional research is required in order to understand better how including chickpeas in the diet may affect CVD risk

factors especially in Western populations. The following chapters describe controlled and ad libitum investigations into the effect of chickpeas on physiological and metabolic variables, with particular emphasis on CVD risk factors such as serum lipid profile, glycaemic control and body weight.

1.4 Aim of studies

The aim of the studies described in the following chapters, was to investigate the effect of incorporating chickpeas in a typical 'Australian-style' diet, on some metabolic variables linked to CVD risk. The approach was two-fold:

1. To compare the effect of a chickpea-supplemented intervention diet to an isoenergetic, macronutrient-matched, high-fibre wheat-supplemented intervention on serum lipids and lipoproteins, glycaemic control, satiation and bowel function. To facilitate better understanding of the physiological role of dietary fibre, the study also included a comparison of the effect of the wheat-supplemented intervention diet to an isoenergetic, macronutrient matched, lower-fibre wheat-supplemented diet on bowel function and satiation.
2. To investigate whether results obtained under the conditions of the controlled study would also occur under everyday conditions that were both more realistic and allowed observation of changes in food choice and intake. Observation of a group of free-living adults tracked the ways in which they adapted their habitual, *ad libitum* diet to include a minimum of four cans of chickpeas per week.

Chapter 2, a scientific paper accepted for publication, reports on the aims, methodology, results and discussion of the controlled intervention study. An account of the lipid results of part of this study formed the basis of my Honours thesis. Chapter 3 reports on the aims, methodology, results and discussion of the *ad libitum* study. Some repetition between the chapters is unavoidable, as they have been prepared as scientific papers for submission to peer-reviewed journals. Similarly, the format of chapters 2 and 3 differ slightly, to comply with requirements of the journals selected for publication. Chapter 4 summarises and discusses key findings common to the controlled and *ad libitum* studies as well as suggesting future directions for investigation of the long-term effects of chickpea consumption on CVD risk factors.

Appendix 1, a scientific paper accepted for publication, reports on a larger collaborative study involving two centres - Launceston and Melbourne that included the serum lipid and lipoprotein data of the controlled dietary intervention study described in Chapter 2. All contributors were involved in study design, protocol and revision of the manuscript. The candidate administered the Launceston study, performed and analysed the laboratory and statistical data, wrote the original manuscript and edited subsequent versions. M.J. Ball was the investigator in charge of the Launceston group and approved final manuscript; I.K. Robertson was the consultant biostatistician; K.D.K. Ahuja assisted in collection of data from the Launceston group, laboratory testing and statistical analysis and P.J.Nestel was the investigator in charge of Melbourne group. Reference to this paper appears in the General Discussion (Chapter 4).

1.5 Design of studies

Participants with a personal and/or family history of CVD risk factors such as age (>40 years), heart disease, high blood pressure or high cholesterol were targeted for the studies, to increase the possibility of a mildly hypercholesterolaemic/insulin resistant test population. Some authors have reported increased sensitivity to cholesterol-lowering intervention diets in hypercholesterolaemic individuals (5, 35, 48, 138, 175) and postulated enhanced response to low GI diets in hyperglycaemic individuals (121).

A random crossover design with a washout period in between was chosen to compare the effects of the controlled intervention diets in the first study. The crossover design utilizes participants as their own controls, thereby reducing the effect of genetic variability – a confounding factor in intervention studies. In addition, a crossover design reduces the number of participants required to detect a difference in outcome - if there is one to detect. Randomly assigning participants to either diet initially, helps reduce bias that may influence the outcome of the study eg dietary compliance may be better during the initial dietary phase than the second, thus if diets were assigned in fixed sequence, some difference in results may be due to compliance, not to diet or carry-over. The inclusion of a wash-out period, where participants return to their usual diet, helps prevent any carry-over effect from the first diet to the next and allows any blood parameters that have been altered to return to pre-study levels prior to the commencement of the second dietary phase (96).

The ad libitum study followed an ordered crossover design, including two periods of habitual ad libitum dietary intake before and after the chickpea-supplemented phase. When one phase of dietary comparison is the habitual diet of the participants, it is not possible to randomly assign dietary phases or include a washout period. The ordered crossover design chosen allows for two periods of habitual dietary phase to help control for any adjustments participants may make to their diet as a consequence of participating in a dietary study.

It has been reported to take four weeks for blood lipid levels to reflect the effects of altered dietary intake where isocaloric conditions prevail (145, 146). The dietary intervention periods in the controlled and ad libitum studies were of at least five weeks duration. This allowed participants to acclimatize to the increased presence of chickpeas in their diet. The washout period in the controlled study was of six weeks duration. The chickpea phase of the ad libitum study was of 12 weeks duration to facilitate detection of potential change in anthropometric measures such as body weight, BMI, waist and hip circumference.

Due to time constraints, only twelve of the twenty-one participants were able to commit to the third, lower-fibre wheat-based dietary phase of the controlled study. Again, due to time constraints, the lower-fibre dietary phase was of only three weeks duration and was commenced directly after the completion of the second phase. However, we were confident that three weeks was

enough time to reflect any changes to bowel function or satiation due to the change in dietary intervention.

Four-day weighed dietary records, which included two week-end days, were used to determine energy intake initially and in the first and fifth week of each controlled dietary phase. Dietary records are more accurate than food frequency questionnaires for assessing dietary consumption of individuals or small groups (144). A pitfall of all recording of dietary intake is under reporting and dietary intake being affected by the act of recording (113). It has been reported that energy intake calculated from four-day dietary records is comparable to that calculated from seven-day records, when two week-end days are included (41). It has also been reported that calculations of energy intake from seven-day dietary data is not significantly different to calculations made using data collected daily for one year (80). During the chickpea phase of the ad libitum study, seven-day dietary records allowed researchers to analyse how consumption of chickpeas during the course of a week altered participants' habitual food intake.

The 'gold standard' method for measurement of peripheral tissue sensitivity to insulin is the euglycaemic insulin clamp, however the test is very invasive and lengthy (60). The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) is a validated index for calculation of basal insulin resistance (or peripheral tissue insulin sensitivity) (60, 107, 109) using fasting plasma glucose and insulin concentrations:

$$\text{HOMA-IR} = \text{fasting insulin } (\mu\text{IU/ml}) \times \text{fasting glucose (mmol/L)} / 22.5.$$

Paired t-tests are the statistical test used when comparing means of paired data i.e. data recorded at two different intervals (eg chickpea diet, wheat diet) on the same individuals. Simple paired t-test cannot be used accurately in this study design because of the need to adjust for the effects of diet order and chronological period. Multiple time points cannot be handled by a simple paired t-test. The number of data points should be the same and follow a normal distribution and statistically, the more sets of data measured, the greater the probability of significant results happening by chance (136, 147).

Repeated measures ANOVA with robust standard error estimation goes some way to alleviating these problems: the method is reliable enough to overcome discrepancies in sample size and minor deviations in the distribution of the differences which may affect the appropriateness of the estimates of confidence intervals. There may also be deviations from the assumptions of the ANOVA methods based on ordinary least squares regression (normality of distribution, lack of heteroskedasticity and missing variables). In addition, ANOVA allows comparison of more than two intervals on more than two populations. General linear modelling (GLM) is a form of maximum likelihood regression analysis; a means of assessing the effect of predictor (independent) variable/s on response (dependent) variable/s. In addition, ANOVA using GLM can analyse the effect of both categorical and continuous variables simultaneously. The effect of one predictor on one response is univariate analysis; the effect of many distinct predictors for each response is termed multivariate analysis (136, 147). Thus repeated

measures ANOVA using GLM was used, because it is a reliable method that accounts for the effect of diet order and chronological period of measurement of two or more dietary periods and can analyse the effect of individual nutritional predictor variables and combinations, on specific response variables such as serum TC and LDL-C.

The rank order (or ordinal) data obtained from the bowel function and satiety questionnaires must be [measured] with other methods. We used the Wilcoxon's signed rank test and Proportional Odds Modelling (POM) by ordinal logistic regression for repeated measures as rank order equivalents of paired t-test and GLM respectively.

(Thank you to Dr. Iain Robertson for assisting with the statistics explanation).

Chapter 2

Effects of a Controlled Diet Supplemented with Chickpeas on Serum Lipids, Glucose Tolerance, Satiation and Bowel Function

2.1 Abstract

Objective: To compare the effect of a diet supplemented with chickpeas to a wheat-based diet of similar fibre content on serum lipids, glucose tolerance, satiation and bowel function. A third, lower-fibre wheat diet provided further information on the effects of dietary fibre quantity on bowel function and satiation.

Method: Twenty-seven free-living adults completed a randomised crossover study comprising two controlled dietary interventions each of five weeks duration. The chickpea diet included canned drained chickpeas, bread and shortbread biscuits containing 30% chickpea flour. The wheat diet included high-fibre wheat breakfast cereals and wholemeal bread. The diets were isoenergetic to the participants' usual diet, matched for macronutrient content and controlled for dietary fibre. Following on from the second randomised intervention, a sub-group of 18 participants underwent a third, isoenergetic lower-fibre wheat diet that included low-fibre breakfast cereals and bread.

Results: Repeated measures ANOVA revealed reductions in serum TC of 0.25 mmol/L ($p < 0.01$) and LDL-C of 0.20 mmol/L ($p = 0.02$) following the chickpea diet compared to the wheat. An unintended, significant increase in PUFA and corresponding decrease in MUFA consumption occurred during

the chickpea diet and statistical adjustment for this reduced but did not eliminate the effect on serum lipids. There was no significant difference in glycaemic control. Perceived general bowel health improved significantly during the chickpea diet although there was considerable individual variation. Some participants reported higher satiation during the chickpea diet.

Conclusions: The small but significant decrease in serum TC and LDL-C during the chickpea diet compared to the equivalent fibre wheat diet was partly due to unintentional changes in macronutrient intake occurring because of chickpea ingestion. If dietary energy and macronutrients were not controlled, chickpea consumption might result in greater benefits via influence on these factors.

2.2 Introduction

Examination of the literature reveals a gradual development of interest in the contribution of pulses to a healthy lifestyle, as awareness of ethnic diets and lifestyles has grown (16, 17, 30, 40, 85). Chickpeas are a common component of traditional diets of Asian, Mediterranean, Arab and South American communities (48, 130). In contrast to most other pulses and cereals, chickpeas have a relatively high fat content (115, 172, 184); however, the fat is composed mostly of polyunsaturated fatty acids (PUFA), with less than 1% saturated fatty acids (SFA). Although chickpeas contain less carbohydrate than, for example, wheat (172), the starch contained has a higher amylose content (30-40% compared to 20%) (53, 121, 184) and the amylose has a greater degree of polymerisation (1667 glucose residues compared to 540) (135). This renders chickpea starch more resistant to

digestion in the small intestine, resulting in lower bioavailability of glucose (116, 121, 135) and higher availability of substrate for colonic fermentation – contributing to improved bowel health and synthesis of short chain fatty acids (53, 135).

Compared to cereal grains, legumes overall are a very good source of dietary fibre (167, 175). Dietary fibre includes resistant starch, non-starch polysaccharide (cellulose, hemicellulose, pectin, gums and β -glucans), non-digestible oligosaccharides and lignin (43, 47, 69, 103, 167). Dietary fibre can be differentiated into soluble (pectin, gums and β -glucans) and insoluble fibre (cellulose, hemicellulose, non-digestible oligosaccharides and lignin) (69, 103, 167). While the ratio of soluble to insoluble fibre in legumes is comparable to grains (approximately 1:3 for both) (175) per 100g edible portion, chickpeas contain 17.4g total dietary fibre compared to 12.7g for wheat (172).

Increased consumption of soluble, viscous fibre has been associated with decreased serum total cholesterol (TC), decreased serum low density lipoprotein-cholesterol (LDL-C) and inversely correlated with coronary heart disease (CHD) mortality rates (7, 43, 69, 90, 103, 125). The association between increased consumption of insoluble fibre and reduced risk of CHD is not as strong as with soluble fibre (125, 128). Higher consumption of dietary fibre, in particular resistant starch, has been associated with improved glycaemic control and insulin sensitivity (69, 71, 167). Dietary fibre may also be beneficial in the fight against obesity. It has been suggested that a state of

high satiation may be reached faster and last longer after ingestion of higher fibre foods because they are bulkier and take longer to eat than lower fibre foods (24, 103) and delay gastric emptying (19, 69). Increased consumption of dietary fibre has also been associated with improved bowel health and stool consistency (19, 28, 167).

Even though chickpeas are a common constituent of many ethnic diets and are rich in PUFA and dietary fibre – resistant starch in particular, there has been little research into chickpeas and human health compared to other pulses. The focus of the current study was an investigation into the effect of substituting wheat-based foods with chickpeas on serum lipid profiles, long-term glycaemic control, bowel function and satiation. The study compared the results of a chickpea-supplemented dietary intervention (test diet) to an isocaloric wheat-based intervention (control diet), both of five weeks duration. A small, sub-study compared the effects of a three-week, isocaloric, lower-fibre wheat diet to that of the aforementioned wheat-based control diet, to evaluate the effect of amount of fibre as well as source of fibre on bowel health and satiation.

2.3 Materials and Methods

2.3.1 Participants

Adults less than 70 years of age not taking medication for hyperglycaemia or hyperlipidaemia were invited to participate. All participants gave written informed consent and were free to leave the study at any time. The Northern

Tasmanian Health and Medical Human Research Ethics Committee approved the study (application no. H7142).

2.3.2 Study design

The study followed a randomised crossover design using two controlled dietary intervention periods – chickpea or wheat-based, each of five weeks duration. A washout-period of six to eight weeks separated the two dietary periods, during which time participants resumed their normal diet. Following on from the second randomised dietary phase, some participants commenced a third lower-fibre wheat-based dietary intervention (lower-fibre diet) of three weeks duration. Twelve participants agreed to have their bowel transit time (BTT) measured during the final week of each dietary period.

2.3.3 Diet design

Prior to commencing the study, participants weighed and recorded four days of 'usual' dietary intake, which was analysed using Foodworks 2.1 computer software (Xyris, Brisbane, Australia). This 'usual' record helped formulate individual isoenergetic chickpea and wheat intervention diets. The diets were comparable in energy, protein, carbohydrate, total fat and dietary fibre – except for the lower-fibre diet, which contained approximately half the amount of dietary fibre as the wheat. Every effort was made to maintain consistent consumption of type and quantity of dietary fats (oil, spread, cheese, milk, yoghurt, ice cream) during each phase. Four-day records of weighed dietary intake were analysed to determine participant nutrient intake for each dietary period.

Participants refrained from eating any foods with cholesterol-lowering claims, (e.g. margarine containing phytosterols), legumes (other than the chickpeas supplied) or foods with high fibre claims (e.g. 'fibre enriched' yogurt or fruit juices) and maintained their usual pattern of physical activity throughout the study period.

Chickpea diet (test diet): This intervention was based on the daily consumption of 140g of canned, drained chickpeas (Edgell 300g net weight, Simplot Australia) plus bread and shortbread biscuits – made with 30% chickpea flour.

Wheat diet (control diet): This intervention was based on the daily consumption of wholemeal (wheat) bread and higher wheat fibre breakfast cereals (> 3.0g fibre /100g).

Lower-fibre wheat diet: Designed to provide comparative information on bowel function, utilised white bread and lower wheat fibre breakfast cereals (< 3.0 g/100 g).

2.3.4 Questionnaires

Participants completed questionnaires concerning stool consistency, bowel function and satiation after the first and final week of each dietary intervention. Visual analogue scales (150 mm) anchored with descriptors aided assessment of frequency and ease of defecation, frequency of

flatulence, perceived bowel health and satiation. To determine stool consistency, participants referred to the Bristol Stool Form Scale (93).

2.3.5 Laboratory measurements

Collection of venous blood samples followed overnight fasting for ten hours. Serum and plasma aliquots were stored at -70 °C until analysis. Serum TC, triacylglycerols, high-density lipoprotein cholesterol (HDL-C) and plasma glucose were assayed in the same run for each participant, using an RA 1000 auto analyser (Technicon, USA) and ThermoTrace reagents (Thermo Electron Corporation, USA). Friedewald's equation was used to calculate LDL-C (45). Serum insulin was measured using Insulin Radioimmunoassay Kits (Diagnostic Systems Laboratories Inc., Australia) and an LKB multi gamma counter plus RiaCalc software (Version 3). The homeostasis assessment model of insulin resistance (HOMA-IR) equation was used to calculate basal insulin resistance (107):

$$\text{HOMA-IR} = \text{fasting insulin } (\mu\text{IU/ml}) \times \text{fasting glucose (mmol/L)} / 22.5.$$

2.3.6 Bowel transit time (BTT)

Twelve consenting participants received gelatine capsules containing radio-opaque markers of different sizes and shapes. Ingestion of the markers and subsequent collection of the faecal samples used to ascertain BTT, occurred in the final week of each dietary period. Examination by x-ray determined the number and shape of radio-opaque markers present in each sample.

Calculation of bowel transit time utilised the following equation (133):

$$\text{BTT} = \frac{t_1s_1 + t_2s_2 + t_3s_3}{s_1 + s_2 + s_3}$$

t_1, t_2, t_3 time of ingestion of each capsule

s_1, s_2, s_3 number of each marker present

2.3.7 Statistical Analysis

STATA Statistical Data Analysis, version 8.2 (STATA 8.2 Statacorp, USA) was used for statistical analysis. Repeated measures ANOVA using General Linear Modelling (GLM) was used to compare results for each of the diets and to examine the effect of diet and dietary components on serum lipid profile and glucose tolerance. Answers from the questionnaires were analysed using Wilcoxon's Signed Rank Test for non-parametric data.

2.4 Results

2.4.1 Subject description

Thirty-one participants commenced the first, randomly assigned, intervention period – either chickpea- or wheat-based. Four participants failed to complete both the diets, three due to employment commitments and one due to discomfort attributed to chickpea consumption. The mean age (\pm SD) of the remaining twenty-seven participants (7 pre-menopausal and 10 post-menopausal women and 10 men) was 50.6 ± 10.5 years and BMI 28.8 ± 4.4 m/kg². The mean fat content of their 'usual' diets was 87.7 ± 28.3 g/day (33% of energy - %E). The mean proportion of SFA was 44 ± 7 % of total fat consumed (%TF), 17 ± 6 %TF PUFA and 39 ± 4 %TF monounsaturated fatty acids (MUFA). The amount of dietary fibre consumed was 27.9 ± 7.1 g/day with 48% provided by the cereal food group, 26% from vegetables, 19% from

fruit and 7% from nuts and legumes. Eighteen participants (2 pre menopausal and 9 postmenopausal women and 7 men) agreed to undergo the third, low-fibre wheat diet during which they consumed 15.2 ± 1.60 grams of fibre per day, from the same food sources as above but using lower fibre choices.

Table 2.1 Comparison of nutritional intake and bodyweight at the end of the dietary periods^{1, 2}

	Dietary periods	
	Wheat	Chickpea
Body weight (kg)	83.8 (77.3 to 90.4)	83.9 (77.4 to 90.4)
Total energy consumed (MJ/day)	9.08 (8.48 to 9.69)	8.89 (8.35 to 9.42)
Protein (%E) ³	18.2 (17.0 to 19.5) ^a	17.2 (16.1 to 18.2) ^b
Carbohydrate (%E)	42.6 (40.5 to 44.6)	43.6 (41.4 to 45.8)
Total fat (%E)	34.0 (32.1 to 36.0)	33.9 (31.8 to 35.9)
Saturated fat (%TF) ³	40.4 (37.5 to 43.2)	40.5 (37.2 to 43.8)
Polyunsaturated fat (%TF)	14.7 (13.4 to 16.0) ^a	17.6 (15.7 to 19.4) ^b
Monounsaturated fat (%TF)	45.0 (42.6 to 47.4) ^a	42.0 (38.8 to 45.1) ^b
Dietary fibre (g/day)	29.3 (26.3 to 32.2)	28.4 (26.4 to 30.5)

1 Values are mean (95% Confidence Interval)

2 n=27

3 Values expressed as a percentage of energy (%E) or as a percentage of total fat (%TF) consumed

a or b Different superscripts denote significant difference ($p < 0.05$) (repeated measures analysis of variance using general linear modelling)

2.4.2 Nutrient content of diets

During the chickpea intervention, chickpea based foods contributed approximately 2.4 MJ of energy per day from protein (17%E), total fat (22%E), carbohydrate (61%E) and approximately 15g of dietary fibre. During the wheat intervention, wheat based foods contributed approximately 2.6 MJ of energy per day from protein (14%E), total fat (16%E), carbohydrate (70%E) and approximately 17g of dietary fibre.

Table 2.1 shows the mean nutrient intake as recorded by study participants in the final week of the chickpea- and wheat-based diets. Similarity in body weight at the end of each diet suggests total macronutrient intake was comparable. There was a significant decrease in mean consumption of protein, as a percentage of energy consumed, during the chickpea diet even though the difference was only one percent of energy consumed ($p=0.04$). This difference was due to substitution of meat-group foods with chickpeas during the chickpea phase. The mean intake of protein contributed by meat, fish, poultry or eggs was 89 g/day (28%) compared to 140 g/day (40%) during the wheat diet. Analysis of the background diets of the participants during each intervention i.e. nutrient intake apart from wheat or chickpea-based foods (Table 2.2), also showed a significant decrease in consumption of protein (%E) with a corresponding increase in carbohydrate consumption during the chickpea diet compared to the wheat ($p=0.001$). Otherwise the background diets of the participants during the interventions were comparable.

Total fat consumption was similar during the intervention diets, as designed; however, fatty acid consumption was unexpectedly, significantly different. Participants consumed significantly more polyunsaturated fatty acids (PUFA) during the chickpea diet compared to the wheat ($p<0.01$) and less monounsaturated fatty acids (MUFA) ($p=0.03$).

Table 2.2 Comparison of background nutritional intake (apart from chickpea and wheat products) at the end of the dietary periods ^{1,2}

	Dietary periods	
	Wheat	Chickpea
Total energy consumed (MJ/day)	6.6 (5.91 to 7.25)	6.4 (5.83 to 6.96)
Protein (%E) ³	20.1 (18.4 to 21.9) ^a	17.3 (15.7 to 18.8) ^b
Carbohydrate (%E)	35.5 (32.2 to 38.8) ^a	39.3 (36.3 to 42.4) ^b
Total fat (%E)	39.4 (36.6 to 42.1)	38.4 (35.8 to 40.9)
Saturated fat (%TF) ³	40.4 (37.5 to 43.3)	40.2 (36.6 to 43.9)
Polyunsaturated fat (%TF)	12.8 (11.1 to 14.5)	14.2 (12.2 to 16.2)
Monounsaturated fat (%TF)	46.8 (44.4 to 49.1)	45.6 (41.8 to 49.3)
Dietary fibre (g/day)	13.3 (10.8 to 15.9)	12.4 (10.8 to 14.1)

¹ Values are mean (95% Confidence Interval) ² n=27

³ Values expressed as a percentage of energy (%E) or as a percentage of total fat (%TF) consumed per day

^a or ^b Different superscripts denote significant difference ($p<0.05$) (repeated measures analysis of variance using general linear modelling)

2.4.3 Effect of chickpea diet on serum lipids and glycaemic response

Table 2.3 shows the results of serum lipid profiles, glucose, insulin and insulin resistance (HOMA-IR score), for the chickpea- and wheat-based diets, adjusted for order of diet and chronological period of measurement. There was a significant reduction in mean serum TC of 0.25 mmol/L ($p < 0.01$) and LDL-C of 0.20 mmol/L ($p = 0.02$) during the chickpea diet compared to the wheat. Results for glucose, insulin and HOMA-IR were not significantly different.

Table 2.3 Comparison of results for each dietary intervention phase ^{1,2}

	Wheat	Chickpea
Bowel transit time (hrs) ³	41.7 (29.0 to 54.5) ^a	52.3 (39.0 to 65.7) ^b
TC (mmol/L) ⁴	6.13 (5.62 to 6.65) ^a	5.88 (5.36 to 6.39) ^b
LDL-C (mmol/L) ⁴	4.09 (3.65 to 4.52) ^a	3.89 (3.45 to 4.33) ^b
HDL-C (mmol/L) ⁴	1.36 (1.21 to 1.50)	1.33 (1.19 to 1.47)
Triacylglycerols (mmol/L) ⁴	1.53 (1.31 to 1.75)	1.44 (1.14 to 1.75)
Glucose (mmol/L) ⁴	5.33 (5.04 to 5.62)	5.26 (5.02 to 5.51)
Insulin (μ U/ml) ⁴	9.33 (6.89 to 11.77)	9.87 (7.38 to 12.35)
HOMA-IR ₄ , ⁶	2.23 (1.54 to 2.92)	2.33 (1.68 to 3.00)
	Wheat	Low fibre wheat
Bowel transit time (hrs) ⁵	39.9 (27.3 to 52.5) ^a	48.7 (29.5 to 68.0) ^b

¹ Values adjusted for order of diet and chronological order of measurement

² Values are mean (95% Confidence Interval) ³ n=12 ⁴ n=27 ⁵ n=10

⁶ Homeostasis Model of Assessment of Insulin Resistance

^a or ^b Different superscripts denote significant difference ($p < 0.05$)

(repeated measures analysis of variance using general linear modelling)

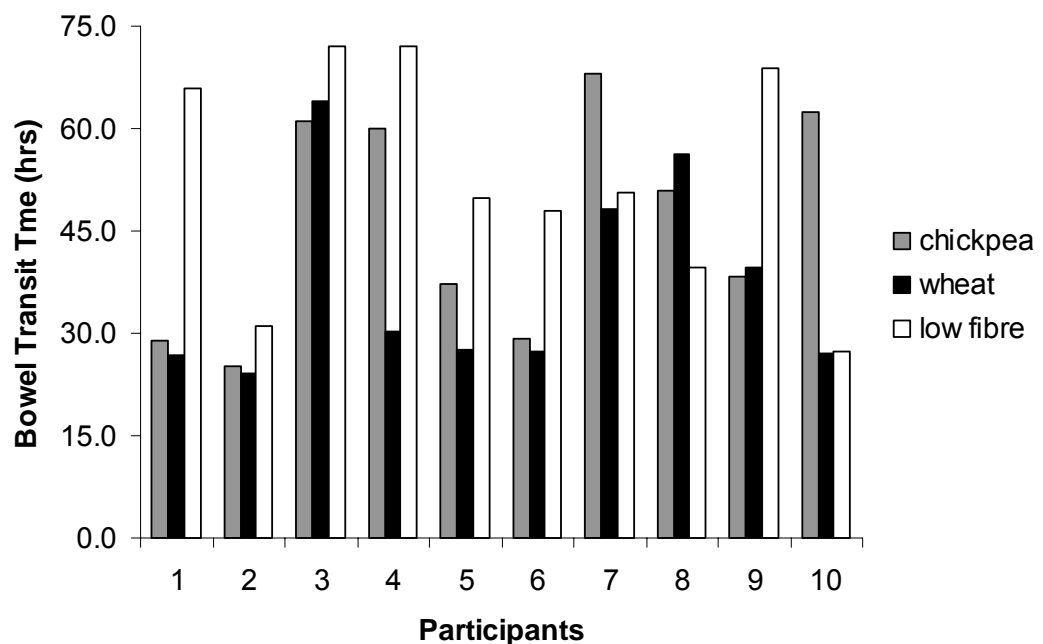
Univariate analysis (adjusted for order of diet and chronological period of measurement) suggested that PUFA and MUFA were the dietary components that produced the greatest singular effect on serum TC and LDL-C. With each increase in standard deviation of PUFA ingested, mean serum TC reduced by 0.29 mmol/L and LDL-C by 0.24 mmol/L. Conversely, with each increase in standard deviation of MUFA ingested, mean serum TC increased by 0.20mmol/L and LDL-C by 0.13 mmol/L. Dietary fibre had only a marginal effect, with mean serum TC and LDL-C both reduced by 0.04 mmol/L for every increase in standard deviation of dietary fibre.

The diets were then classified into those components that the subjects were advised to include in their diet in order to create the differences between the diets (the chickpea- and wheat-based foods), and those components that were common to both diets (the background diets). The effect of the chickpea versus the wheat diet was again compared, this time after adjustment of the common diet for PUFA and fibre content. The difference in mean serum TC was reduced on the chickpea diet from 0.25 mmol/L ($p<0.01$) to 0.12 mmol/L ($p=0.18$) and mean LDL-C from 0.20mmol/L ($p=0.02$) to 0.07 mmol/L ($p=0.27$) by adjustment for PUFA. The singular effect of PUFA on serum TC and LDL-C was again much greater than the effect of dietary fibre. This time, one standard deviation increase in PUFA was associated with a mean decrease in serum LDL-C of 0.26mmol/L, compared to a mean decrease of 0.05 mmol/L for one standard deviation increase in dietary fibre.

2.4.4 Effect of diet on BTT and appreciation of bowel function by participants

Table 2.3 also shows the results of BTT for the intervention diets, adjusted for order of diet and chronological period of measurement. For the chickpea-wheat comparison, results for eight of the 12 participants showed very little difference in BTT due to diet, while results for four individuals were markedly longer during the chickpea diet compared to the wheat. Consequently, the mean BTT was 10.6 hrs longer during the chickpea diet compared to the wheat ($p=0.02$). For the higher-lower wheat fibre comparison, the mean BTT was 8.8 hrs longer during the lower-wheat fibre diet compared to the higher ($p=0.03$), with the majority of participants showing a longer BTT during the lower-wheat fibre dietary period. Figure 2.1 demonstrates the wide variability of results from participants who underwent a BTT during each of the three dietary periods.

Fig 2 1. Comparison of bowel transit times between the chickpea, wheat and low-fibre wheat diets for each participant (n=10)



The questionnaire responses for the chickpea-wheat comparison showed a great deal of variation and thus fewer significant differences were detected. During the chickpea diet, participants recorded a variety of effects on stool consistency in week one (hard to mushy) which resolved to smooth and soft by week five. There was a slight trend in defecation becoming easier during the course of both diets while frequency of defecation increased slightly during the wheat diet and decreased slightly during the chickpea diet. Just under half the respondents noticed an initial increase in the frequency of flatulence for both diets, the remainder reporting no change. Perceived bowel health was initially significantly better during the chickpea diet compared to the wheat (chickpea vs. wheat week 1; $p=0.04$) and improved significantly over the course of the chickpea diet (chickpea week 1 vs. chickpea week 5; $p=0.04$). One individual (dashed line) reported 'terrible' bowel health throughout the wheat diet compared with 'much better' health during the chickpea phase. Even so, there was no significant difference in perceived bowel health between the chickpea and wheat diets in week 5, due to the wide variation in individual responses. The degree of satiation reported by the group was high throughout both the dietary periods.

For the higher-lower wheat fibre subgroup comparison, participants recorded a variety of effects on stool consistency (hard to mushy) throughout each of the dietary periods. There were no significant differences detected in frequency of defecation or perceived bowel health – the participant who reported 'terrible' bowel health during the wheat phase of the chickpea-wheat comparison did not undertake the lower-fibre wheat diet. Ease of defecation

was slightly reduced from usual during the lower-fibre wheat diet and marginally easier during the higher-fibre wheat but no significant difference was detected ($p=0.06$). Frequency of flatulence on the other hand, was reported as significantly greater during the higher-fibre wheat diet in week 5 ($p=0.02$). While the degree of satiation did not alter during the lower-fibre wheat diet, again, satiation was significantly higher during the higher-fibre wheat diet, after both one and five weeks ($p\leq 0.01$).

2.5 Discussion

Significant reductions in serum TC and LDL-C followed five weeks consumption of a chickpea-supplemented test diet compared to a wheat-based control diet of similar dietary fibre content. Statistical analysis suggested that an unanticipated change in fatty acid composition during the chickpea diet (particularly PUFA) was related to the reductions in serum TC and LDL-C. Adjusting the data to take account of the effect of PUFA substantially reduced, but did not abolish, the difference in serum TC and LDL-C between the chickpea and wheat diets. This suggests some other component or components of chickpeas were responsible for 40% of the effect of the chickpea diet on serum TC and LDL-C. A meta-analysis investigating the hypocholesterolaemic effect of non-soy pulses on serum lipids (9) concluded that while soluble dietary fibre contributed the greatest effect, other factors such as oligosaccharides, isoflavones, phospholipids and fatty acids, phytosterols, saponins, other vitamins and minerals also played an important role. Even so, the authors concluded it was the sum of the

whole rather than individual components that were responsible for the hypocholesterolaemic effect of pulses.

The absence of observed effect of dietary fibre on serum TC and LDL-C may have been due to the similar dietary fibre content of the chickpea and wheat diets (28.73 vs. 27.86 g/day) coupled with the similar ratio of soluble to insoluble fibre present in chickpeas and wheat. Furthermore, the dietary fibre intake during the chickpea and wheat diets was very similar to the mean 'usual' intake of this group of participants (27.9 ± 7.1 g/day). While a number of studies have investigated the effect of high fibre in addition to other dietary components on glucose tolerance and hyperlipidaemia (6, 11, 46, 155) (99) (127) only a few have investigated the effect of high dietary fibre intake alone (26). All of these studies compared intervention diets containing at least two to three times more dietary fibre (primarily insoluble fibre) than the control or usual diet - in some cases, five or six times greater (11, 46, 155). It has been suggested that cholesterol-lowering by high fibre diets is best observed in studies where the dietary fibre intake is very high (25), as much as two to three times the recommended intake (138). In the current study, the focus was to compare the effect of source of dietary fibre rather than quantity. Thus, the chickpea and wheat intervention diets contained a realistic amount of dietary fibre, consistent with recommended dietary guidelines (37), rather than an extreme amount that participants may have found difficult to consume; potentially affecting compliance.

The chickpea intervention did not have any significant effect on glucose tolerance or insulin sensitivity compared to the wheat diet, even though chickpeas contain more resistant starch than wheat. However alterations in dietary fatty acid content between the intervention diets involved adjustment to the PUFA:MUFA ratio; the P:S ratio remained unaffected. Research suggests substitution of unsaturated fatty acids for SFA may improve insulin sensitivity (66, 67, 148, 183), so substitution of PUFA for MUFA may not be expected to cause any change. Another controlled, dietary intervention study (121) also reported no change in fasting plasma glucose, insulin concentrations or HOMA-IR after six weeks of chickpea-supplemented intervention compared to wheat, even though post-prandial results showed reduced plasma glucose and insulin responses following ingestion of a chickpea-based meal compared to a wheat meal. The authors postulated that the normoglycaemic state of their participant population (5.2 ± 0.4 mmol/L, n=19) might have contributed to the apparent lack of long-term improvement in glucose tolerance. The participant population for the current study would also be considered normoglycaemic, with mean fasting glucose levels below 6.0 mmol/L and insulin concentrations of less than 30 μ IU/ml (39). Furthermore, in both the current study and that of Nestel et al (121) there was no significant weight loss during the dietary intervention periods and there was no change in the P:M:S ratio of the intervention diets of Nestel et al (121).

The current study also surveyed the bowel function and perceived bowel health of participants during the dietary periods. The results suggest that

increased consumption of chickpeas would not adversely affect bowel function compared to increased consumption of wheat. It may prove beneficial for gluten-sensitive individuals looking for alternatives to increase their dietary fibre content. Flatulence has generally been associated with ingestion of pulses (36) but in this study, although a significant reduction in frequency of flatulence was detected in the subgroup during the lower-fibre wheat phase, there was no difference between the chickpea and wheat diets. Chickpeas should thus not be deemed unacceptable for this reason. The canning process and further cooking by participants could have reduced the oligosaccharide activity (and thus degree of flatulence) of the chickpeas.

Research generally supports an inverse relationship between fibre content of the diet and BTT (19, 28, 167). However, focus on the effect of particular fibre sources is not as clear-cut. Addition of wheat bran to the diet has been shown to reduce BTT but this effect may be due to the physical form of the wheat bran used rather than a particular constituent (25, 28, 92, 170). Other studies have reported no change in BTT after ingestion of wheat bran or pectin (161), oat hull fibre (162) or green lentils (163). It has also been suggested that one of the physiological effects of resistant starch is a tendency to increase BTT rather than reducing it (117) Dietary fibre consumption is one of many variables that influence colonic function. Other variables include gender, age, stress, hormones, hydration and the absorptive function of the small intestine (28). Even though in the current study both the chickpea and lower-fibre BTT's were significantly longer than during the control wheat diet, they were still within the normal range of 1-4

days (24–96 hrs) (28). Thus, any difference is difficult to interpret and may just highlight the variation in individual response to dietary change.

The majority of participants had no trouble adjusting to the chickpea diet and there was high acceptance of both the chickpea bread and shortbread biscuits. Most appetites were satisfied during the chickpea and wheat diets. The significant reduction in satiation noted during the lower-wheat fibre phase may have been due to an unplanned reduction in energy consumption during this intervention where, to keep dietary fibre to a minimum, participants consumed white rice rather than pasta or potato along with lower fibre fruit and vegetables. Some participants commented that during the chickpea diet they no longer 'craved' the sweet and fatty 'treats' to which they were normally 'addicted'. This fits with comments to the American Dietetics Association that the less quantifiable effects of dietary fibre such as satiation are just as important as the statistically significant effects of fibre consumption (103).

2.6 Conclusion

Substitution of chickpeas for wheat-based foods in a controlled dietary intervention resulted in small but significant reductions in serum TC and LDL-C that were partly due to changes in fatty acid content. Chickpeas as a whole may have contributed a small benefit - both in their own right and/or through dietary substitution. Chickpeas are rich in PUFA and may have provided improvements related to this. In addition, inclusion of chickpeas may have caused other beneficial physiological and dietary changes associated with

increased satiation. These aspects need to be explored in studies that do not attempt to control the overall macronutrient or non-chickpea fibre content of the intervention diets, as this study indicates that more beneficial physiological and biochemical changes may result via this mechanism.

Chapter 3

Chickpeas influence P:S ratio and fibre content of *ad libitum* dietary intake leading to improved serum lipid profile, glycaemic control and satiation.

3.1 Abstract

Objective To estimate the effect of including a realistic quantity of chickpeas in an otherwise *ad libitum* diet of free-living adults.

Design Ordered crossover design of 20 weeks duration.

Subjects Forty-five adult women and men, as a group slightly hypercholesterolaemic but normoglycaemic.

Intervention Participants included a minimum of four, 300 g cans of chickpeas per week in their habitual diet for twelve weeks.

Main outcome measures Comparison of macronutrient and dietary fibre consumption, body weight, body mass index, fasting plasma glucose, serum lipids, lipoproteins, insulin, leptin and ghrelin concentrations, after habitual diet supplemented with chickpeas and after four weeks of post chickpea *ad libitum* diet. Semi-quantitative assessment of bowel function and satiation using anchored visual analogue scales.

Statistical analyses Repeated measures ANOVA using General Linear Modelling (GLM) with robust standard error estimation. Ordinal logistic regression for ordinal data.

Results Chickpea-related increases in mean dietary fibre and polyunsaturated fatty acids intake were associated with decreases in serum total and low-density lipoprotein cholesterol, fasting insulin and HOMA-IR when compared to the usual dietary phase. Small but significant reductions in body weight with increased perceived satiation and improved bowel function were noted during the chickpea phase compared to the usual dietary phase.

Conclusions Adding chickpeas to the diet is an option for individuals wanting to modify their diet associated CVD risk factors.

3.2 Introduction

The association between diet and cardiovascular disease (CVD) is well known (1, 2, 66, 110, 143). Any uncertainty concerns the choice of replacement macronutrient/s for dietary saturated fatty acids (SFA) that will achieve optimal glycaemic control and blood lipid concentrations and thus greatest reduction in CVD risk (66, 143). Candidate macronutrients include poly- and monounsaturated fatty acids (PUFA & MUFA), high-fibre wholegrain carbohydrates and plant proteins. In addition to CVD, obesity is associated with hypertension, insulin resistance, non-insulin dependent diabetes mellitus, sleep apnoea, (84) some cancers, osteoarthritis, gall bladder disease (178) gout, mood and eating disorders (20, 55). Modest weight loss (~10%) has been shown to improve glycaemic response, insulin sensitivity and lipid profile (58, 59, 82, 102, 173, 174). Advances in

understanding the metabolic interactions that govern energy balance, appetite and body weight have fuelled increased interest in the effect of diet and dietary constituents on satiation (degree of fullness leading to meal cessation), satiety (interval between cessation of one meal and initiation of the next) (62, 137, 188) and biomarkers of energy regulation such as leptin and ghrelin (31, 137, 158). Some researchers suggest diets rich in complex carbohydrate (dietary fibre) and low in fat have a greater satiating effect than diets high fat and low in carbohydrate (61, 158, 188) and thereby contribute to reduced energy consumption and weight loss. It has been suggested that satiation may be reached faster and satiety last longer after ingestion of higher-fibre, complex carbohydrate-containing foods because they are bulkier and take longer to eat than lower fibre foods (63, 103) and delay gastric emptying (19, 63, 69). Both ghrelin, an appetite stimulant strongly associated with hunger ratings and satiety and leptin, an appetite suppressant associated with long-term energy balance, are more strongly affected by dietary carbohydrate than dietary fat (31). Increased consumption of higher fibre foods has also been associated with improved bowel health and stool consistency (29). (28, 167).

Chickpeas are a rich source of unsaturated fatty acids – particularly PUFA (115, 172, 184), resistant starch (53, 121, 184), dietary fibre (172) vitamins (51), minerals (114, 129) and phytoestrogens (108, 118, 149, 151, 154).

They are relatively free of antinutrients, such as lectins, but do contain small amounts of saponins, some tannins and phytate (51, 129, 134).

Previous controlled dietary intervention studies performed by the current authors (Appendix 1 and Chapter 2), suggested that isoenergetic chickpea supplementation of an equivalent-fibre wheat-based 'Australian' style diet, brought about increased PUFA consumption that was related to small but significant reductions in serum low-density lipoprotein (LDL) and total cholesterol (TC) concentrations, in mildly hypercholesterolaemic women and men. However, macronutrient control of the diets could have reduced the impact of chickpea consumption. The purpose of the current study was to observe the effects of chickpea supplementation on *ad libitum* nutrient intake, serum lipids, lipoproteins and other metabolic and physiological changes in a more realistic setting. Apart from consumption of a minimum amount of canned chickpeas per week for 12 weeks, participants were under no other dietary constraints.

3.3 Method

3.3.1 Participants

Adults aged of 30 and 70 years, not on medication to control hyperglycaemia or hyperlipidaemia, were recruited via the local media. Criteria for acceptance into the study included the presence of one or more cardiovascular disease (CVD) risk factors likely to result in dyslipidaemia or poor glucose tolerance (e.g. elevated serum total cholesterol, overweight, older age) and/or a family history of heart disease, non-insulin dependent diabetes or CVD risk factors. Participants gave written informed consent and were free to leave the study at any time. The Northern Tasmanian Health and Medical Human Research Ethics Committee granted approval for the study (application no. H0007926).

3.3.2 Study design

Participants commenced the dietary phases in an ordered crossover fashion, beginning with a four-week period of their habitual *ad libitum* dietary intake (familiarisation phase), followed by twelve weeks of *ad libitum* dietary intake including chickpeas (chickpea phase) and then a second four week phase of their habitual *ad libitum* dietary intake (usual phase). When one phase of dietary comparison is the habitual diet of the participants, it is not possible to blind the dietary phases or randomly assign them. The ordered crossover design chosen allows for two periods of habitual dietary phase to help control for any adjustments participants may make to their diet as a consequence of participating in a dietary study. Twelve weeks of chickpea inclusion allowed for longer-term observation of possible dietary change and incremental change in body weight. Time considerations prevented a 12-week usual phase being practical and a four-week phase allowed sufficient time for observation of any metabolic changes.

Using Terrillon 'Café Inox' food-weighing scales (2 g), supplied for the study, participants weighed and recorded their dietary intake during the final week of each dietary phase and half way through the chickpea phase.

Analysis of these records checked compliance with chickpea consumption and determined nutrient consumption during the chickpea and usual phases. This helped to determine which foods the chickpeas were replacing.

The participants completed questionnaires concerning stool consistency, bowel function and satiation during the final week of the chickpea and usual phases and at the midpoint of the chickpea phase. Visual analogue scales (150 mm) anchored with descriptors aided assessment of frequency and ease of defecation, frequency of flatulence, perceived bowel health and satiation. To determine stool consistency, participants referred to the Bristol Stool Form Scale (93).

3.3.3 Diet design

During the familiarisation and usual phases, participants were requested to consume their habitual *ad libitum* dietary intake. During the chickpea phase participants were required to incorporate a minimum of four, 300 g (net weight) cans of chickpeas (Edgell, Simplot, Australia) per week into their *ad libitum* diet. Per 100 g, the chickpeas provided 0.47 MJ of energy, 14.8 g of carbohydrate, 6.8 g of protein, 1.2 g of total fat – 0.3 g SFA, 0.7 g MUFA, 0.8 g PUFA, 6.9 g of dietary fibre – 6.2 g insoluble and 0.7 g soluble. The researchers provided the cans, and a selection of recipes - to equip participants with a variety of alternatives for chickpea consumption. No advice was given to the participants regarding chickpea food exchange; they were allowed to make their own adjustments, as one of the aims of the study was observing the effect of chickpea supplementation on *ad libitum* nutrient intake.

3.3.4 Laboratory measurements

Collection of venous blood samples followed ten hours of overnight fasting. Samples for serum collection were stored at room temperature until coagulation and clot retraction had occurred; samples for plasma isolation were stored on ice until separation. Serum and plasma aliquots were obtained by centrifugation at 4 °C for 20 minutes at 2500 rpm. Aliquots were stored at -70 °C. Prior to testing, the serum samples were thawed at room temperature and thoroughly mixed.

Serum TC, triacylglycerols, high-density lipoprotein cholesterol (HDL-C) and plasma glucose were assayed in the same runs for each participant to reduce the inter-assay variability, using a DataPro Clinical Analyser (Thermo Trace Ltd, USA) and ThermoTrace reagent kits (Thermo Trace Ltd, USA). Mean laboratory intra-assay precision for plasma glucose, serum TC, triacylglycerols and HDL-C was 1.9%, 2.1%, 2.3% and 5.1% respectively. Friedewald's equation (45) was used to calculate serum LDL-C and the homeostasis assessment model of insulin resistance (HOMA-IR) equation (107) was used to calculate basal insulin resistance (peripheral tissue insulin sensitivity):

$$\text{HOMA-IR} = \text{fasting insulin } (\mu\text{U/ml}) \times \text{fasting glucose (mmol/L)} / 22.5.$$

Serum insulin was measured using Insulin Radioimmunoassay Kits (Diagnostic Systems Laboratories Inc., Texas, USA); serum leptin was measured using Active Human Leptin Immunoradiometric Assay Kits (Diagnostic Systems Laboratories Inc., Texas, USA); serum total ghrelin was

measured using Ghrelin (total) Radio Immunoassay kits (LINCO Research, Missouri, USA) and an LKB multi gamma counter plus RiaCalc software (Version 3). The sensitivity of the methods at the 95% confidence level was 1.3 μ IU/ml for insulin, 10 ng/ml for leptin and 93 μ g/ml for ghrelin, according to information supplied with the reagent kits. Intra-assay coefficients of variation for serum insulin, leptin and ghrelin were 8.0%, 2.1% and 5.6% respectively.

3.3.5 Statistical analysis

Sample size calculation based on previous studies in similar populations (13) indicated that 45 subjects would be required to detect a 5% reduction in total cholesterol concentration using a cross-over design (standard deviation of change in cholesterol of 0.9 mmol/L, power 80%, alpha 0.05). STATA Statistical Data Analysis, version 8.2 (Statacorp, USA) was used to analyse the data. Repeated measures ANOVA using General Linear Modelling (GLM) with robust standard error estimation was used to examine the effect of diet and dietary components on serum lipid profile and glycaemic control. Responses to the bowel function and satiation questionnaires were analysed using Proportional Odds Modelling (POM) by ordinal logistic regression for repeated measures ordinal outcome data.

3.4 Results

3.4.1 Effect of Chickpea supplementation on dietary constituents, physiological and anthropomorphic measures

Fifty participants commenced the familiarisation phase of the study (34 females, 16 males). One male withdrew during this initial phase due to work commitments; two withdrew after six weeks of the chickpea phase, one due to health problems unrelated to chickpea consumption and another following an adverse reaction to venipuncture. Two females withdrew after six weeks of the chickpea phase, one due to health problems not related to chickpea consumption and one due to a surfeit of chickpeas. The baseline characteristics of the 45 participants who completed the twenty-week study (32 females, 13 males) are contained in Table 3.1. The group was mildly hypercholesterolaemic but normoglycaemic.

Table 3.2 compares the mean daily macronutrient and dietary fibre intake of the participants during the final week of each of the dietary periods: the familiarisation phase - just prior to the inclusion of chickpeas in their diets; the chickpea phase – after 16 weeks of including chickpeas in their diet and the usual phase, four weeks after the participants had returned to their habitual ad libitum diet. Statistically significant alterations in dietary intake during the familiarisation and usual phases compared to the chickpea phase are indicated by differing superscripts. These differences are more clearly illustrated in table 3.3.

Table 3.1. Baseline characteristics of the study participants ¹

		Mean ± sd
Age	years	52.2 ± 6.09
Weight	kg	74.7 ± 16.0
Body Mass Index	kg/m ²	26.3 ± 4.82
Waist Hip Ratio	ratio	0.84 ± 0.09
Total Cholesterol	mmol/L	6.51± 1.39
HDL-Cholesterol	mmol/L	1.68 ± 0.39
LDL-Cholesterol	mmol/L	4.10 ± 1.05
Triacylglycerols	mmol/L	1.52 ± 1.11
TC:HDL	ratio	4.02 ± 0.97
HDL:LDL	ratio	0.44 ± 0.15
Glucose	mmol/L	4.95 ± 1.00
Insulin	μIU/ml	6.25 ± 4.29
HOMA-IR ²		1.45 ± 1.34

¹ n=45

² Homeostasis model assessment of insulin resistance, a mathematical model of basal insulin sensitivity, derived from fasting glucose and fasting insulin concentrations.

Table 3.2 Dietary intake during the final week of the familiarisation, chickpea and usual dietary phases¹

		Familiarisation phase	Chickpea phase	Usual phase
		Mean ± sd	Mean ± sd	Mean ± sd
Dietary fibre	g	24.31 ± 7.77 ^b	28.60 ± 6.97 ^a	21.83 ± 8.19 ^b
Total energy	mJ	7.87 ± 1.60	7.85 ± 2.11	7.63 ± 1.81
Protein	%E	18.28 ± 2.93 ^a	18.44 ± 3.26 ^a	17.54 ± 2.45 ^b
Carbohydrate	%E	46.97 ± 7.72	46.73 ± 5.79	46.61 ± 6.55
Total fat	%E	29.71 ± 5.63 ^a	29.75 ± 5.68 ^a	31.36 ± 6.02 ^b
Sfa ²	%TF	42.53 ± 6.11 ^a	41.56 ± 6.33 ^a	44.70 ± 7.84 ^b
Pufa ³	%TF	16.87 ± 4.41 ^b	18.30 ± 4.35 ^a	15.64 ± 5.29 ^b
Mufa ⁴	%TF	40.60 ± 3.58	40.14 ± 3.53	39.67 ± 4.19
P:S ratio	ratio	0.42 ± 0.18 ^b	0.47 ± 0.19 ^a	0.39 ± 0.26 ^b

¹N=42

² Saturated Fatty Acids

³ Polyunsaturated Fatty Acids

⁵ Monounsaturated Fatty Acids

^{a b} Different superscripts denotes a significant difference compared to the chickpea phase, $p < 0.05$ (repeated measures ANOVA using general linear modelling)

Table 3.3 shows the mean differences in quantity of dietary fibre, macronutrients and fatty acids consumed in the first and final weeks of the chickpea and usual phases. Mean dietary fibre intake was increased by 4.30 g/day (95% CI: 2.91 g/day to 5.69 g/day, $p < 0.001$) during the chickpea phase. There was also a significant increase in mean PUFA consumption of 1.43% of total fat (95% CI: 0.28 %TF to 2.58 %TF, $p = 0.02$), which coupled with a small decrease in mean saturated fatty acid (SFA) consumption, caused the P:S ratio to significantly increase during the chickpea phase by 0.05 (95% CI: 0.00 to 0.09, $p = 0.045$). During the usual phase mean PUFA consumption decreased by 2.66 %TF (95% CI: -4.35 %TF to -0.97 %TF, $p < 0.001$) and mean SFA consumption increased by 3.14 %TF (95% CI: 0.97 %TF to 5.30 %TF, $p < 0.001$) resulting in a decrease in the mean P:S ratio of 0.08 (95% CI: -0.15 to 0.00, $p = 0.045$). In addition, there was a significant decrease in mean dietary fibre consumption of 6.77 g/day (95% CI: -8.58 g/day to -4.96 g/day, $p < 0.001$), in mean percent of energy provided by protein of 0.91% (95% CI: -1.74 %E to -0.08 %E, $p = 0.03$) and an increase in energy provided by total fat of 1.62% (95% CI: 0.04 %E to 3.19 %E, $p = 0.05$) during the usual phase.

Table 3.3. Mean difference in dietary components consumed in the first and final weeks of the chickpea and usual dietary phases^{1, 2}

		Chickpea phase	Usual phase
Dietary fibre (g)	Mean change	4.30	-6.77
	95% CI	2.91 to 5.69	-8.58 to -4.96
	P value	<0.001	<0.001
Total energy (MJ)	Mean change	-0.02	-0.23
	95% CI	-0.52 to 0.48	-0.54 to 0.08
	P value	0.93	0.15
Protein (%E)	Mean change	0.16	-0.91
	95% CI	-0.59 to 0.91	-1.74 to -0.08
	P value	0.67	0.03
Total carbohydrate (%E)	Mean change	-0.24	-0.12
	95% CI	-2.08 to 1.60	-1.78 to 1.54
	P value	0.80	0.89
Total fat (%E)	Mean change	0.04	1.62
	95% CI	-1.74 to 1.82	0.04 to 3.19
	P value	0.97	0.05
SFA ³ (%TF)	Mean change	-0.97	3.14
	95% CI	-2.64 to 0.70	0.97 to 5.30
	P value	0.25	<0.001
PUFA ⁴ (%TF)	Mean change	1.43	-2.66
	95% CI	0.28 to 2.58	-4.35 to -0.97
	P value	0.02	<0.001
MUFA ⁵ (%TF)	Mean change	-0.46	-0.47
	95% CI	-1.61 to 0.70	-1.66 to 0.71
	P value	0.44	0.43
P:S ratio	Mean change	0.05	-0.08
	95% CI	0.00 to 0.09	-0.15 to 0.00
	P value	0.045	0.045

¹ N=42

² P<0.05 denotes a significant difference (repeated measures analysis of variance using general linear modelling)

³ Saturated Fatty Acids

⁴ Polyunsaturated Fatty Acids

⁵ Monounsaturated Fatty Acids

Table 3.4 shows the difference in mean anthropometric and laboratory measurements recorded at the beginning and end of dietary phase. During the familiarisation phase, there were small but significant reductions in mean body weight of 0.54 kg (95% CI: -0.85 kg to -0.24 kg; $p=0.001$), in BMI of 0.19 kg/m² (95% CI: -0.30 kg/m² to -0.09 kg/m²; $p=0.001$), in serum TC of 0.18 mmol/L (95% CI: -0.35 mmol/L to -0.01 mmol/L; $p=0.04$), in fasting insulin of 1.11 μ U/ml (95% CI: -1.87 μ U/ml to -0.34 μ U/ml; $p=0.01$) and in HOMA-IR of 0.32 (95% CI: -0.55 to -0.09; $p=0.01$). There was a small reduction of borderline significance in body weight during the chickpea phase of 0.42 kg (95% CI: -0.87 kg to 0.03 kg; $p=0.07$) and in BMI of 0.16 kg/m² (95% CI: -0.23 kg/m² to 0.01 kg/m²; $p=0.07$). No other changes in anthropometric or laboratory measures were detected, even after adjustment for age, gender and BMI. During the usual phase, all values increased, with significant increases in mean serum TC of 0.20 mmol/L (95% CI: 0.08 mmol/L to 0.33 mmol/L, $p=0.002$), in LDL-cholesterol of 0.19 mmol/L (95% CI: 0.05 mmol/L to 0.33 mmol/L, $p=0.01$), in fasting insulin of 0.75 μ U/ml (95% CI: 0.02 μ U/ml to 1.48 μ U/ml; $p=0.045$) and HOMA-IR of 0.21 (95% CI: 0.05 to 0.37; $p=0.01$).

Table 3.4. Mean difference in anthropometric and laboratory measurements recorded at the beginning and end of each dietary phase ^{1, 2}

		Familiarisation phase	Chickpea phase	Usual phase
Body weight (kg)	Mean change	-0.54	-0.42	0.03
	95% CI	-0.85 to -0.24	-0.87 to 0.03	-0.51 to 0.58
	P value	0.001	0.07	0.90
Body mass index (kg/m ²)	Mean change	-0.19	-0.16	0.01
	95% CI	-0.30 to -0.09	-0.32 to 0.01	-0.19 to 0.21
	P value	<0.001	0.06	0.94
Waist hip ratio	Mean change	0.00	0.00	0.00
	95% CI	-0.01 to 0.01	-0.01 to 0.01	-0.01 to 0.01
	P value	0.54	0.84	0.54
Total cholesterol (mmol/L)	Mean change	-0.18	-0.07	0.20
	95% CI	-0.35 to -0.01	-0.23 to 0.09	0.08 to 0.33
	P value	0.04	0.37	0.002
LDL-Cholesterol (mmol/L)	Mean change	-0.11	-0.05	0.19
	95% CI	-0.27 to 0.06	0.25 to 0.15	0.05 to 0.33
	P value	0.20	0.64	0.01
HDL-Cholesterol (mmol/L)	Mean change	-0.03	-0.01	0.01
	95% CI	-0.07 to 0.01	-0.06 to 0.03	-0.04 to 0.05
	P value	0.13	0.53	0.79
Triacylglycerols (mmol/L)	Mean change	-0.01	-0.09	0.11
	95% CI	-0.17 to 0.14	-0.30 to 0.13	-0.06 to 0.28
	P value	0.88	0.44	0.20
Glucose (mmol/L)	Mean change	-0.10	-0.04	0.18
	95% CI	-0.29 to 0.10	-0.26 to 0.19	-0.03 to 0.39
	P value	0.33	0.75	0.09
Insulin (μ U/ml)	Mean change	-1.11	0.39	0.75
	95% CI	-1.87 to -0.34	-0.35 to 1.14	0.02 to 1.48
	P value	0.01	0.30	0.05
HOMA-IR ³	Mean change	-0.32	0.09	0.21
	95% CI	-0.55 to -0.09	-0.09 to 0.27	0.05 to 0.37
	P value	0.01	0.31	0.01
Ghrelin (pg/ml)	Mean change		-54.64	
	95% CI		-145.91 to 36.64	
	P value		0.24	
Leptin (μ g/L)	Mean change		0.61	
	95% CI		-1.26 to 2.47	
	P value		0.52	

¹ N=45

² P<0.05 denotes a significant difference (repeated measures analysis of variance using general linear modelling)

³ Homeostasis model assessment of insulin resistance, a mathematical model of basal insulin sensitivity, derived from fasting glucose and fasting insulin concentrations

Univariate analysis investigated the effect of change of diet as well as selected dietary components on serum TC and insulin concentrations (Table 3.5). The change in diet (usual compared to chickpea) had the greatest effect with a mean increase in serum TC of 0.20 mmol/L and in insulin of 0.89 μ IU/ml. Of the individual dietary components, dietary fibre had greatest effect on serum TC, while PUFA and SFA had equivalent but opposing effects on serum TC and insulin and protein had a small effect on insulin. With each one standard deviation mean increase of dietary fibre consumed, mean serum TC decreased by 0.41 mmol/L; with each one standard deviation mean increase in PUFA consumed, mean serum TC decreased by 0.30 mmol/L and insulin by 0.70 μ IU/ml. Conversely, with each one standard deviation mean increase in SFA, mean serum TC increased by 0.26 mmol/L and insulin by 0.75 μ IU/ml. When multivariate repeated-measures ANOVA was used to adjust for the effect of selected macronutrients that differed between the dietary phases the apparent independent effect of the usual diet was substantially reduced. Multivariate regression is unable, however, to separate the effects of non-independent variables, such as when the quantity of chickpeas and the intake of PUFA or dietary fibre or protein are measures of the same real thing, or when an increase in PUFA occurs with an equivalent decrease in SFA.

Table 3.5. Individual effect of Usual versus Chickpea dietary phases and dietary components on serum TC and insulin ¹

	Total Cholesterol (mmol/L)			Insulin (μ IU/ml)		
	Mean difference ²	95% CI	P value	Mean difference	95% CI	P value
Usual minus Chickpea	0.20	0.06 to 0.33	0.01	0.89	0.14 to 1.64	0.02
Protein %E (z-score ³) (mean 17.96 \pm sd 2.85)	0.12	-0.15 to 0.39	0.38	-0.51	-1.05 to 0.04	0.07
Carbohydrate %E (z-score) (mean 46.78 \pm sd 6.51)	-0.10	-0.39 to 0.19	0.50	1.05	-0.22 to 2.30	0.10
Total fat %E (z-score) (mean 30.44 \pm sd 5.69)	0.05	-0.22 to 0.32	0.72	-0.47	-1.56 to 0.62	0.40
SFA ⁴ %TF (z-score) (mean 42.57 \pm sd 7.15)	0.26	0.00 to 0.52	0.046	0.75	0.17 to 1.33	0.01
PUFA ⁵ %TF (z-score) (mean 17.31 \pm sd 5.21)	-0.30	-0.53 to -0.08	0.01	-0.70	-1.31 to -0.10	0.02
MUFA ⁶ %TF (z-score) (mean 41.12 \pm sd 4.10)	-0.08	-0.33 to 0.17	0.53	-0.42	-0.99 to 0.15	0.15
Dietary fibre g (z-score) (mean 25.89 \pm sd 8.02)	-0.41	-0.73 to -0.09	0.01	0.33	-1.53 to 2.19	0.73

¹ Repeated measures Analysis of variance using general linear modelling for each variable in the table modelled separately; p<0.05 denotes significant effect

² Mean difference for Diet is the mean difference in serum total cholesterol (mmol/L) and fasting insulin (μ IU/ml) measured at the end of the chickpea and usual dietary phases. For the dietary components, it is the effect of an increase of one standard deviation in the covariant on the mean difference in serum total cholesterol (mmol/L) and fasting insulin (μ IU/ml) measured at the end of the chickpea and usual dietary phases.

³ Standardised normal value [= (subject variable value – group mean)/ standard deviation] calculated for each subject

⁴ Saturated fatty acids

⁵ Polyunsaturated fatty acids

⁶ Monounsaturated fatty acids

Mean chickpea consumption was significantly increased during the chickpea phase by 119 g/day. Adjusting for chickpea content revealed that for every one standard deviation increase in mean chickpea content there was a corresponding increase in mean energy supplied by protein of 0.57 %E (95% CI: 0.01 %E to 1.12 %E; p=0.046) and a significant increase in mean dietary fibre consumption of 3.40 g (95% CI: 1.79 g to 5.0 g, p<0.001). Mean PUFA and MUFA as a percent of total fat (%TF) also increased with increasing chickpea content, whereas mean SFA (%TF) decreased however, none of the fatty acid changes were statistically significant. Thus, adding chickpeas to the diets significantly increased the energy supplied by protein and dietary fibre content while contributing to a decrease in energy supplied by total fat – particularly SFA. Further investigation of foods the chickpeas were substituting indicated that there was reduced consumption of all food-types during the chickpea phase. In particular, mean cereal intake reduced by 44.6 g/day and vegetables by 37.0 g/day. During the usual phase, consumption of dairy foods increased significantly by 50 g/day and highly processed snack food consumption increased by 42.6 g/day.

3.4.2 Effect of Chickpea supplementation on satiation and perceived bowel function

Questionnaire analysis (Table 3.6) revealed a significant increase in frequency of flatulence during the chickpea phase with a three-fold decrease during the usual phase. Perceived general bowel health remained consistent throughout the study and there was no change in frequency of defecation. Stool consistency did not change significantly during the chickpea phase but

participants reported significantly harder stools during the usual phase and defecation required more effort. Participants reported significantly higher satiation during the chickpea phase.

Table 3.6. Mean difference in bowel function and satiation measured during the first and final weeks of the chickpea and usual phases ^{1,2}

		Chickpea phase	Usual phase
Stool consistency	Mean change	0.51	-0.55
	95% CI	-0.11 to 1.12	-1.08 to -0.03
	P value	0.11	0.04
Ease of defecation	Mean change	1.52	-2.04
	95% CI	0.81 to 2.23	-2.89 to -1.19
	P value	<0.001	<0.001
Frequency of defecation	Mean change	0.65	-0.77
	95% CI	-0.24 to 1.54	-1.67 to 0.14
	P value	0.35	0.10
Frequency of flatulence	Mean change	0.74	-2.13
	95% CI	0.02 to 1.47	-3.26 to -1.01
	P value	0.04	<0.001
Perceived general bowel health	Mean change	0.54	-0.39
	95% CI	-0.28 to 1.36	-1.43 to 0.65
	P value	0.20	0.47
Satiation	Mean change	1.49	-1.76
	95% CI	0.68 to 2.30	-2.52 to -1.00
	P value	<0.001	<0.001

¹ N=42

² P<0.05 denotes a significant difference (proportional odds modelling by ordinal logistic regression for repeated measures ordinal outcome data)

3.5 Discussion

Including four cans of chickpeas per week for 12 weeks, in the *ad libitum* diet of 45 free-living, healthy adults, resulted in small but significant increases in mean dietary fibre and PUFA consumption and increased P:S ratio. While these changes were not sufficient to affect serum lipid profile, glycaemic control, or quantitative measures of satiation or weight loss, small reductions of borderline statistical significance in mean body weight and BMI were observed. When participants resumed their usual *ad libitum* diet after completing the chickpea phase, small but significant increases were observed in mean serum TC, LDL-C, fasting insulin and HOMA-IR. These changes coincided with decreased consumption of dietary fibre, protein and PUFA and increased consumption of total fat as SFA, resulting in a decreased P:S ratio. Small but significant reductions in mean body weight, BMI, serum TC, fasting insulin and HOMA-IR during the familiarisation phase, suggests that participants were already making healthy dietary changes before commencement of the chickpea phase. The changes in laboratory and anthropometric measures that occurred during the four-week familiarisation phase were consolidated and improved upon during the twelve-week chickpea phase, only to return to baseline levels during the four-week usual phase. This suggests the usual phase was probably closer to the participants' true habitual eating pattern than the familiarisation phase.

It has been suggested that increased dietary SFA impairs both hepatic LDL-C and glucose clearance and peripheral tissue insulin sensitivity (164, 177) while replacement of SFA with PUFA (183) or MUFA (67) reverses this

effect. Dietary protein also has an inverse association with insulin sensitivity (91, 164). Univariate analysis in the current study, suggested an association between serum TC concentration and PUFA, SFA and dietary fibre content and an association between insulin concentration and dietary PUFA, SFA and protein content. However, as multivariate analysis could not discern the effect of PUFA, SFA, protein or dietary fibre independently of the effect of chickpeas as a whole, it cannot be determined whether the chickpeas themselves caused these changes, the foods they were associated with or the foods they replaced.

During the chickpea phase, chickpeas replaced a wide variety of foods from all food groups but in particular the cereals, pasta group and the vegetable group – both of which include pulses (37). This substitution fitted with the observation of greater PUFA and dietary fibre consumption during the chickpea phase, as chickpeas contain more fibre and PUFA per 100g than wheat (172). Perusal of micronutrient data recorded at the beginning and end of the chickpea phase, revealed a small but statistically significant increase in mean iron intake and a decrease in mean intake of niacin and potassium. Even so, dietary intake for niacin and potassium during the chickpea phase was still above the National Health and Medical Research Council 1991 recommended dietary intakes for Australians (under revision) (123). Adjusting for chickpea content suggested chickpeas contributed added protein and dietary fibre during the chickpea phase but there was no significant association between chickpeas and added PUFA. Thus, the increased PUFA consumption may have been due to a combination of added

chickpea plus the method of preparation i.e. added PUFA-rich cooking oil or dressing. During the usual phase, serves of dairy foods and highly processed 'snack' foods increased significantly, probably explaining the increased consumption of total fat and SFA at the expense of PUFA, protein and dietary fibre.

Altered dietary fibre intake may also have been associated with qualitative changes in satiation during the study (61, 69, 103, 158, 188) and may have contributed to small but significant changes in mean body weight and BMI observed during the study. Even though perceived satiation increased during the chickpea phase, there was no significant decrease in mean ghrelin concentrations. Moreover, even though there was a decrease in mean body weight there was no significant increase in mean leptin concentration. The lack of significant change in leptin and ghrelin concentrations may have been partly influenced by consistent intake of total carbohydrate during the study (31). Furthermore, the very small reduction in body weight during the chickpea phase (0.45 kg over 12 weeks) may have either been physiologically not significant - as suggested by a steady WHR, or resulted in leptin changes too small to be statistically significant.

It has been suggested that cholesterol lowering by high fibre diets is best observed in studies where the dietary fibre intake is very high (25, 72, 138). Thus in this study, neither the 4.3g/day increase during the chickpea phase nor the 6.8g/day decrease during the usual phase, would have been sufficient to cause any significant effect on serum lipid profile due solely to

modified dietary fibre intake. Nevertheless, this may have been sufficient to affect satiation, faecal bulking and ease of defecation. Three studies investigating the effect of small to moderate increases in dietary fibre (3-17g of fibre per day) reported increased faecal bulking but no effect on serum lipid profile (29). (163) (162). Frequency of flatulence rose marginally during the chickpea phase ($p=0.046$) but fell markedly during the usual phase ($p<0.001$). This suggests participants were experiencing increased frequency of flatulence during the familiarisation phase compared to the usual phase with increased dietary fibre the most probable cause (36). During a previous controlled study by the current authors (Chapter 2), frequency of flatulence did not differ between the high-fibre wheat-based diet and a chickpea-supplemented diet of similar dietary fibre content but was significantly higher than during a lower-fibre wheat-based diet. The authors concluded the change in amount of dietary fibre was responsible rather than its source.

3.6 Summary and Conclusion

Including chickpeas in the habitual *ad libitum* intake of 45 healthy participants for 12 weeks resulted in a small but significant increase in mean PUFA and dietary fibre intake and a small but statistically significant reduction in mean body weight. Participants also reported increased satiation during the chickpea phase although this was not borne out by quantitative analysis.

Healthy dietary changes made by participants in the weeks prior to the chickpea phase were associated with small but significant decreases in serum TC, fasting insulin concentrations and HOMA-IR. During the chickpea

phase, these parameters reduced still further but returned to pre-study concentrations on resumption of the usual diet. Whether these findings would translate to smaller or greater effects in a more hypercholesterolaemic and/or hyperglycaemic population is a subject for future study. Results of the current ad libitum study support the findings of our previous controlled studies (Appendix 1, and Chapter 2), suggesting that chickpeas could be a healthy inclusion to the 'Australian-style' diet.

Chapter 4

General Discussion

As mentioned in the discussion sections of Chapters 2 and 3 (and Appendix 1), it was found that changes in fatty acid consumption, increased dietary fibre intake, satiation (degree of fullness leading to meal cessation) and improved bowel function were common outcomes of chickpea-supplementation of an 'Australian style' diet. These findings were independent of study location, study design or length of dietary intervention. During the collaborative study investigating the effect of chickpea consumption on serum lipid profiles (Appendix 1), the Melbourne group consumed more carbohydrate and dietary fibre and less total fat during the six-week chickpea intervention, perhaps 'reflecting changes in food item selection by the participants' (121). Even so, while MUFA and SFA (%TF) consumption was reduced, PUFA consumption remained relatively constant. During the controlled study (Chapter 2) consumption of PUFA increased and consumption of MUFA decreased during the five-week controlled chickpea phase but SFA (and total fat) consumption remained constant. Increased frequency of flatulence was associated with fibre content rather than fibre source, bowel health was not adversely affected during the chickpea intervention and satiation was high. The 12-week chickpea phase of the ad libitum study (Chapter 3) revealed increased consumption of PUFA and dietary fibre, which decreased during the usual phase. Bowel function was again significantly improved and reported satiation was higher during the

chickpea phase compared to the usual diet. Statistical analysis suggested that the small but significant increases in mean PUFA consumption was the dietary component most consistently associated with small but significant reductions in mean serum LDL-C and TC, (with little effect on HDL-C or triacylglycerols) observed after the chickpea phases compared to the controlled wheat and usual ad libitum phases. However, multivariate analysis could not separate the effect of PUFA from the overall effect of PUFA -rich chickpeas. During the usual phase of the ad libitum study, small but significant increases in fasting insulin concentrations and HOMA-IR were most strongly associated with increased mean SFA and concomitant decreased PUFA intake. Again, multivariate analysis could not distinguish between the overall effect of chickpeas and PUFA (or SFA) concentration on changes in glycaemic control. During the controlled study, no such changes in insulin or HOMA-IR were detected, probably because the fatty acid substitution was PUFA for MUFA while SFA intake remained constant.

A meta-analysis of 224 dietary intervention studies conducted between 1966 and 1994 (64), reported that change in SFA and PUFA intake, and thus the P:S ratio, were still the most robust predictors of change in LDL-C, regardless of 'interactions of dietary factors, initial dietary intakes and serum concentrations, study and subject characteristics'. Multivariate analysis of a meta-analysis of 395 metabolic ward studies involving 129 groups of participants conducted between 1962 and 1995 (27), reported that isocaloric increases in PUFA (5 %E) at the expense of carbohydrate, were associated with significantly reduced

blood TC and LDL-C while MUFA showed no significant effect and SFA significantly increased both LDL-C and HDL-C. The effect of SFA was about twice as strong as that of PUFA. These associations persisted regardless of gender, age, BMI or habitual energy intake. It needs to be noted however, that the type of carbohydrate used could influence its effects on serum lipid profile (7, 67, 69, 103, 125, 128). In the same meta-analysis, isocaloric replacement of SFA (10 %E) with unsaturated fats (5 %E MUFA plus 5 %E PUFA) was reported to be three times more effective at reducing blood TC than isocaloric replacement of total fat with complex carbohydrate and did not affect HDL-C concentrations. It was emphasised that while isocaloric substitution of SFA by PUFA (< 10 %E) or MUFA may improve lipid profiles, it would not help control obesity but evidence from more recent studies indicates otherwise. Recent reviews suggest that dietary PUFA in particular may exert a direct effect on lipogenesis in the liver especially, by promoting fatty acid β -oxidation and suppressing lipid synthesis via regulation of peroxisome proliferator-activated receptors (PPAR α & PPAR γ) (3, 141).

An increase in reported satiation during the higher fibre interventions of the controlled dietary studies had suggested that in ad libitum environments chickpea consumption might contribute to reduced dietary intake and perhaps weight loss. This did happen, but only to a very small extent. During the chickpea phase of the ad libitum study, participants very successfully incorporated chickpeas into their habitual dietary pattern with very little change

in energy intake. Even so, there was a small but significant increase in mean dietary fibre intake and a small but significant decrease in mean body weight, compared to the usual phase. The change in body weight may have been too small to elicit a significant change in leptin concentration, or may not have been physiologically significant. It is noted that there was a lack of change in WHR. The absence of significant change in ghrelin concentrations during the chickpea phase may have been influenced by the consistent intake of total carbohydrate during the study (31) and supported the earlier finding that reported high satiation was due to increased dietary fibre intake (Chapter 2).

The subsequent dietary adjustments observed during the chickpea phase revealed an overall substitution of all food-types with chickpeas, although the food groups most affected were those in which pulses are included.

Micronutrient intake did not seem to be adversely affected by chickpea substitution, although more detailed investigations involving biomarkers of micronutrient absorption would need to be performed to substantiate this and monitor any possible effects on blood levels. Less consumption of dairy and high fat snack foods occurred during the chickpea phase compared to the usual phase. This is consistent with participants' comments of eating fewer between meal snacks during the chickpea phases of the controlled and ad libitum studies.

Another consistent finding during these studies was the relative ease with which participants adjusted to the chickpea interventions - whether they were of five- or 12-weeks duration. The degree of support and encouragement provided by the

investigators along with an extensive range of recipes and preparation ideas, to help participants incorporate the chickpeas into their diets, would have contributed. The majority of participants reported no ill effects from chickpea consumption, apart from increased flatulence, although a number reported feeling very full and bloated during the initial weeks of the ad libitum chickpea phase. Once participants had adjusted their dietary intake to accommodate the chickpeas, acceptance was very high. Of the 102 participants who commenced the studies, only three of the ten withdrawals cited chickpea-associated issues as the reason for their withdrawal.

There has been little research into chickpeas and human health compared to that involving other pulses. Previous research has determined the glycaemic index of chickpeas (73, 101); investigated the effect of chickpea consumption on serum lipid profile compared to other hypocholesterolaemic agents (49) and after ingestion of a high SFA diet (106) and investigated the effect of chickpea consumption on glycaemic control (121). The intention of the current studies was not only to add to this small body of research but also to create practical, real-life scenarios that would be easily attainable for individuals interested in reducing their diet-related CVD risk factors. Participants with greater likelihood of having CVD risk factors (40 years or older, overweight, mildly hypercholesterolaemic/ hyperglycaemic) but otherwise healthy were invited to participate. In addition, it was hoped that changes to metabolic parameters would be more likely to be detected if the parameters to be measured were initially, mildly elevated. However in practice, a number of the participants did

not have baseline abnormalities. The five-week, controlled, dietary interventions were of sufficient length to allow for stabilisation of metabolic parameters without affecting participant compliance. The 12-week, ad libitum, chickpea phase facilitated detection of potential incremental change in anthropometric measures and allowed observation of dietary substitution due to chickpea supplementation. Consideration of participant compliance also influenced the quantity of chickpeas to be used. Larger quantities of chickpeas may have resulted in greater changes in measured parameters but may have been difficult for participants to consume for the duration of the intervention phases and impractical for longer term consumption. As it was, participants reported initial difficulty in consuming the allocated quantity of chickpeas during both the five- and 12-week phases and took a little time to adjust - especially during the ad libitum diet where dietary adjustment was the responsibility of the participants rather than controlled by the investigators. After 12 weeks (3 months), using the modest quantity we chose, participants were happily adjusted to their changed diet and some were reluctant to return to their former dietary habit.

Overall, reported bowel function either did not change, or improved following chickpea consumption, in particular, ease of defecation and faecal bulking. This small effect may have been because the increase in dietary fibre during the chickpea interventions was relatively small. Again, consumption of larger amounts of chickpeas may have produced larger increases in dietary fibre with greater change in bowel function; however participants may have found such

intervention diets less tolerable with potentially a greater incidence of undesirable side effects.

In summary, addition of chickpeas to an 'Australian-style' diet under easily attainable controlled and ad libitum conditions resulted in small but healthy changes to serum lipid profile, bowel function and satiation. Relatively new biomarkers of satiation and energy balance, ghrelin and leptin, remained unchanged during the ad libitum chickpea phase, even though small borderline significant reductions in body weight were observed. This may have been due to consistent carbohydrate intake throughout the study. Participant compliance was high but they received a lot of support and information. Informing and educating the general population about incorporation of chickpeas into their diets would be a challenge but may facilitate greater awareness and acceptance of chickpeas and other pulses as healthy additions to the Australian diet.

Future directions for investigation of the long-term effect of chickpeas on CVD risk factors may sensibly focus on the effect of chickpea intervention in more obese, hyperglycaemic, hypercholesterolaemic populations and in studies designed to incorporating a weight-reducing dietary phase. The challenge would be to fashion diets that had minimal effect on leptin concentrations while suppressing ghrelin concentrations, thus increasing satiation and minimising appetite stimulation while maximising weight loss. The consistent reports of increased satiation also suggest further research is warranted into the effects of chickpeas on post-prandial glycaemic response and between-meal satiety.

Appendix 1

Dietary supplementation with chickpeas for at least five weeks results in small but significant reductions in serum total- and LDL-cholesterol in adult women and men.

J.K. Pittaway, K.D.K. Ahuja, I.K. Robertson, P.J. Nestel, M.J. Ball

Abstract

Objective: To compare the effect of a chickpea-supplemented diet with a wheat-supplemented diet on human serum lipids and lipoproteins.

Method: Forty-seven free-living adults participated in a randomised crossover weight maintenance dietary intervention involving two dietary periods (chickpea-supplemented and wheat-supplemented) each of at least five weeks duration each.

Results: Serum total cholesterol (TC) and low-density-lipoprotein cholesterol (LDL-C) were significantly lower (both $p < 0.01$) by 3.9% and 4.6% respectively, after the chickpea-supplemented diet compared to the wheat-supplemented diet. Protein (0.9% of energy, $p = 0.01$) and monounsaturated fat (3.3% of total fat, $p < 0.001$) intake was slightly but significantly lower and carbohydrate intake was significantly higher (1.7% of energy, $p < 0.001$) on the chickpea-supplemented diet compared to the wheat-supplemented diet. Multivariate analyses suggested

that the differences in serum lipids were mainly due to small differences in polyunsaturated fatty acid and dietary fibre between the two intervention diets.

Conclusions: Inclusion of chickpeas in an intervention diet results in a lower serum TC and LDL-C compared to a wheat-supplemented diet.

Introduction

Research has indicated a strong association between dietary patterns and cardiovascular disease (CVD) (70). A high intake of saturated fats and a low intake of dietary fibre have been strongly associated with high blood lipids (7, 70, 90, 111), a prominent risk factor for CVD (2, 189). Legumes and pulses are low in saturated fat and higher in polyunsaturated fat, protein and complex carbohydrates. They are a good source of resistant starch, soluble and insoluble dietary fibre (48, 129, 134, 172). Examination of the literature reveals a gradual development of interest in the contribution of legumes and pulses to a healthy lifestyle (17, 40). In human studies, consumption of pulses has been associated with reduction of hypercholesterolaemia (5, 8, 119) and reduced risk of coronary heart disease (CHD) (17).

Chickpeas have been a staple part of Indian, Mediterranean and African diets for many thousands of years (48, 160). Worldwide, they are the second most cultivated pulse worldwide and the third largest in terms of the amount of pulse produced worldwide (129, 157, 191). They are, however, a relatively novel addition to Western cuisine. Every 100g of dried chickpeas contain 19.3g of protein, which compares favourably with wheat at 10.7g (172). In contrast to

most other pulses and cereals, chickpeas have a relatively high fat content. This makes them an important energy source for vegans and those without regular access to meat and dairy products - the fat is mostly polyunsaturated, with less than 1% saturated fat (172). While the ratio of soluble to insoluble fibre in legumes is comparable to grains (approximately 1:3 for both) (175) per 100g edible portion, chickpeas contain 17.4g total dietary fibre compared to 12.7g for wheat (172). Chickpeas are a rich source of vitamins, minerals and phytoestrogens. They are relatively free of antinutrients, such as lectins, but do contain small amounts of saponins, some tannins and phytate (51, 129, 134).

There are only two studies which have examined the potential benefits of chickpeas on human health, especially with regard to hypercholesterolaemia (49, 106). Additional research is required to gain a better understanding of the effects of chickpeas in the human diet, especially on CVD risk factors. Thus, the aim of this research was to compare the effects of a chickpea-supplemented diet with the effects of a wheat-supplemented diet on serum lipids and lipoproteins. The latter diet was chosen as a comparison because the 'usual Australian' diet is based on food products made from wheat, e.g. bread, breakfast cereals and pasta. This manuscript presents results obtained from two very similar studies undertaken in Launceston (Tasmania) and Melbourne (Victoria).

Participants and Methods

Participants and Study Design

Free-living adults aged 30 to 70 years, not taking cholesterol-lowering medication were invited to participate. Each participant provided signed, informed consent. The Northern Tasmania Health and Medical Human Research Ethics Committee (Launceston, Tasmania) and the Alfred Research and Ethics Unit (Melbourne Victoria) granted approval for the studies. The studies followed a randomized crossover design, with two periods of dietary intervention - a chickpea-supplemented (chickpea) diet and a wheat-supplemented (wheat) diet. The Launceston group had dietary intervention periods of five weeks duration, separated by an eight-week period over the Christmas recess, when food intake and lifestyle is usually different. The Melbourne group had intervention periods of six weeks duration without an intervening period (121), as they commenced in June and completed the study before Christmas. The comparison was of dietary intakes and blood lipid concentrations at the end of the chickpea-supplemented and wheat-supplemented diets. It did not monitor the changes from a 'baseline' value influenced by uncontrolled usual diet. It has been reported that it takes four weeks for blood lipid levels to reflect the effects of altered dietary intake where iso-energetic conditions prevail (145, 146), and the intervention periods were a minimum of five weeks to allow stabilisation.

Dietary Design

Before starting the dietary intervention periods, participants were asked to record their complete food consumption over four days (two week days and two weekend days) in their weighed food diet diaries, which were used to calculate

usual dietary energy intake using Food Works version 2.10 with the Nuttab 95 and AusNut database (Xyris software, Brisbane, Australia). The intervention diets (chickpea and wheat) were then devised to be iso-energetic with and based on the participants' habitual diet. An attempt was also made to match the total fat, carbohydrate, protein and dietary fibre intake between the two intervention periods.

The chickpea diet involved daily consumption of 140 g (2 serves) of canned, drained chickpeas, chickpea bread and chickpea shortbread biscuits. The chickpeas (Edgell, Australia, 300g net weight) from the same date/batch of canning were provided to the participants. Bread and the shortbread biscuits containing 30% chickpea flour were provided by the Grain Research Development Corporation (Canberra, Australia). This ensured the intake of the same type and variety of chickpeas and chickpea products between participants in the two research centres. Chickpea and chickpea based foods (bread and biscuit) contributed approximately 3.4 MJ of energy per day with 16% of energy from protein, 19% of energy from total fats, and 65% of energy from carbohydrates and approximately 27g of dietary fibre. The wheat diet involved consumption of wholemeal (wheat) bread, high fibre (wheat) breakfast cereals (>2.5g fibre/100g) and shortbread biscuits that participants purchased from their usual grocery suppliers.

Participants were provided with a list of the amount and variety of foods allowed during the two dietary periods. Fruit and vegetable and fat intake was kept similar between the two intervention periods. Participants were requested to refrain from eating any foods with cholesterol-lowering claims, (e.g. phytosterol margarine), legumes (other than the chickpeas supplied) or foods with high fibre claims (e.g. 'fibre enriched' yogurt or fruit juices). Participants were advised not to take more than two standard drinks of alcohol per day. Participants were also asked to maintain their usual pattern of physical activity and body weight throughout the study period.

Participants were contacted regularly to discuss any problems related to diets and to provide encouragement and support. Participants again recorded their four days of dietary intake in the last week of the two intervention diets. These were analysed using FoodWorks software (Xyris, Brisbane, Australia) to calculate and compare nutrient intake between the two intervention diets and to check the dietary compliance.

Laboratory measurements and Statistics

Venous blood samples at the start and the end (day 36 for Launceston Group and day 43 for the Melbourne group) of the two dietary periods were collected at rest, after an overnight fast of ten hours. For serum separation, the blood was allowed to coagulate for one hour and then centrifuged at 800g at 4°C for 20 minutes. Serum was aliquotted and stored at -70°C, for later analysis. All

biochemical analyses were subsequently performed in the same run, to reduce inter-assay variability.

Lipid measurements were performed in complete runs for each participant using an autoanalyser. In Launceston this was an RA 1000 auto analyser (Technicon, USA) and ThermoTrace reagents (Thermo Electron Corporation, USA), whilst in Melbourne a Roche Hitachi 917 autoanalyser and Roche reagents (Roche Diagnostics, Australia) was used. LDL-C for both groups was determined using Friedwald's equation (45)

Repeated measures ANOVA (STATA version 8.2, StataCorp, College Station, Texas USA) performed by general linear modelling (GLM) was used to compare the ingestion of nutrients during the chickpea and the wheat-supplemented diets and to determine the effect of diet on the serum lipids and lipoproteins.

Univariate and multivariate analyses were used to assess the associations between the dietary intakes and lipid profiles. All data were adjusted for the order of diet (chickpea then wheat or wheat then chickpea) and the chronological order of blood sample collection (order and period effects). The results were expressed as the effect size. For categorical variables such as the diet (chickpea or wheat), the effect size was the difference in cholesterol levels (mmol/l) between the two group variables. For continuous dietary component variables (such as fibre, PUFA as % of dietary fat), the standard normal value or z-score [= (subject variable value – group mean)/ standard deviation] was

calculated for each dietary variable for each subject. The effect size for continuous variables was the change in cholesterol levels (mmol/l) associated with a rise of one standard deviation in the dietary variable. Data from each study centre were analysed separately and as a combined group.

Results

Fifty-two people commenced the study (Launceston 31; Melbourne 21). Three participants withdrew due to employment commitments and two more due to abdominal discomfort attributed to ingestion of chickpeas. Thus, 47 participants (Launceston 27; Melbourne 20) completed the study: 28 females and 19 males. The mean (\pm SD) age, weight and BMI at the start of the intervention study was 53.0 ± 9.8 ; 79.3 ± 16.3 kg and 27.6 ± 4.1 kg/m² respectively.

There was no significant difference in the body weight and BMI between the start and the end of each dietary period, or the ends of the two intervention diets (all $p > 0.2$). The body weight and BMI at the end of the chickpea diet were 79.1 ± 16.1 kg and 27.1 ± 4.1 kg/m² while that at the end of the wheat diet were 79.0 ± 16.4 kg and 27.1 ± 4.1 kg/m², respectively. Similarly, serum lipids and lipoproteins were not significantly different at the start of the two intervention diets ($p > 0.6$ for serum TC, LDL-C, HDL-C, and triacylglycerol).

Table 1 shows the mean daily energy, macronutrient, and dietary fibre intake of participants from the final week of each dietary phase. Dietary records, participant feedback and a differential count of cans of chickpeas provided,

indicated that participants consumed the requisite amount of chickpea and wheat products.

Launceston group: There was a small but significantly lower intake of protein (1% of energy; $p=0.04$) during the chickpea diet compared to the wheat diet. Furthermore, there was a significantly higher intake of polyunsaturated fatty acids (PUFA) (2.9 % of total fat; $p=0.01$) and a lower intake of monounsaturated fatty acids (MUFA) (3% of total fat; $p=0.03$) during the chickpea diet compared to the wheat.

Melbourne group: A small but statistically significant lower consumption of total fat (2.2% of energy; $p=0.02$), saturated fatty acids (SFA) (2.9% of total fat; $p=0.03$) and MUFA (3.7% of total fat; $p=0.002$) was observed during the chickpea diet compared to the wheat diet. In contrast, there was a significantly higher intake of dietary fibre (7.0 g; $p=0.02$) and carbohydrate (2.7% of energy; $p=0.01$) intake during the chickpea diet compared to the wheat.

Table 1 Daily macronutrient intake of the participants during the chickpea- and wheat-based diets and end-diet body-weights.¹

	Launceston (n=24)		Melbourne (n=19)		Combined (n=43)	
	Chickpea	Wheat	Chickpea	Wheat	Chickpea	Wheat
Body-weight kg	83.9 ± 17.3	83.8 ± 17.3	72.7 ± 12.1	72.5 ± 12.6	79.1 ± 16.1	79.0 ± 16.4
Dietary fibre g	28.4 ± 5.1	29.3 ± 7.4	33.1 ± 8.2 ^a	26.1 ± 13.3	30.5 ± 7.0	27.9 ± 10.4
Total energy intake mJ	8.9 ± 1.3	9.1 ± 1.5	7.4 ± 2.9	7.5 ± 4.0	8.2 ± 2.3	8.4 ± 2.9
Protein %E	17.2 ± 2.7 ^a	18.2 ± 3.2	19.3 ± 2.2	20.0 ± 2.2	18.1 ± 2.7 ^a	19.0 ± 2.9
Carbohydrate %E	43.6 ± 5.5	42.6 ± 5.1	48.7 ± 6.4 ^a	46.0 ± 6.9	45.8 ± 6.3 ^a	44.1 ± 6.1
Total fat %E	33.9 ± 5.2	34.0 ± 4.9	29.7 ± 7.6 ^a	31.9 ± 7.8	32.0 ± 6.6	33.1 ± 6.4
saturated fatty acids %TF	40.5 ± 8.2	40.4 ± 7.2	34.7 ± 6.2 ^a	37.7 ± 6.0	38.0 ± 7.8	39.2 ± 6.7
polyunsaturated fatty acids %TF	17.6 ± 4.6 ^a	14.7 ± 3.2	16.6 ± 5.9	17.4 ± 5.7	17.1 ± 5.2	15.8 ± 4.7
monounsaturated fatty acids %TF	42.0 ± 7.8 ^a	45.0 ± 6.0	31.3 ± 5.0 ^a	35.0 ± 3.5	37.3 ± 8.5 ^a	40.6 ± 7.1

¹ Values are means ± one standard deviation

² Expressed as the percentage of total energy (%E) or total fat (%TF) consumed per day

^a Means differ between chickpea- and wheat-based diets (Repeated measures Analysis of variance, p<0.05)

There was no significant difference in total energy intake of the individual or combined group. The combined group showed a small but statistically significant lower protein intake (0.9% of energy; $p=0.01$) and MUFA intake (3.3% of total fat; $p<0.001$) on the chickpea diet compared to the wheat diet. Conversely, carbohydrate intake was slightly but significantly higher (1.7% of energy; $p=0.02$) on the chickpea diet compared to the wheat diet. Alcohol intake was similar between the two intervention diets ($p=0.49$).

There was no heterogeneity in the effect of diet between the two centres (centre difference TC 0.08, 95% C.I. -0.16 to 0.32, $p=0.51$; LDL-C 0.03, 95% C.I. -0.18 to 0.25, $p=0.77$). Thus analysis to determine the effect of selected dietary components on serum TC and LDL-C was performed on the combined group.

Serum TC was 3.9% lower (0.22 mmol/l; 95% C.I. 0.1 to 0.35; $p=0.001$) and LDL-C was 4.6% lower (0.18 mmol/l; 95% C.I. 0.07 to 0.29; $p=0.002$) at the completion of the chickpea diet compared to the wheat diet for the combined group (Table 2). The Launceston group showed a 4.1% lower ($p=0.01$) TC and 3.0% lower ($p=0.03$) LDL-C after the chickpea diet compared to the wheat diet. For the Melbourne group TC was 3.5% ($p=0.05$) and LDL-C was 4.4% ($p=0.04$) lower after the chickpea-supplemented diet compared to the wheat-supplemented diet. Serum HDL-C and triacylglycerol levels were not significantly different between the two intervention diets (either in the separate or the combined groups).

Table 2 Serum lipid profiles of participants at the end of each dietary period¹

	Launceston (n=27)		Melbourne (n=20)		Combined (n=47)	
	Chickpea	Wheat	Chickpea	Wheat	Chickpea	Wheat
TC ² mmol/L	5.88 (5.36 to 6.39) ^a	6.13 (5.62 to 6.65)	5.58 (5.14 to 6.02) ^a	5.78 (5.35 to 6.17)	5.75 (5.40 to 6.11) ^a	5.98 (5.62 to 6.33)
LDL-C ³ mmol/L	3.89 (3.45 to 4.33) ^a	4.01 (3.65 to 4.52)	3.46 (3.13 to 3.78) ^a	3.62 (3.26 to 3.40)	3.71 (3.41 to 4.01) ^a	3.89 (3.58 to 4.20)
HDL-C ⁴ mmol/L	1.33 (1.19 to 1.47)	1.36 (1.21 to 1.50)	1.46 (1.26 to 1.67)	1.49 (1.29 to 1.68)	1.39 (1.27 to 1.51)	1.41 (1.29 to 1.53)
Tri ⁵ mmol/L	1.44 (1.14 to 1.75)	1.53 (1.31 to 1.75)	1.47 (0.85 to 2.09)	1.46 (1.03 to 1.89)	1.46 (1.15 to 1.76)	1.50 (1.28 to 1.72)

¹ Values are means with (95% confidence intervals) adjusted for order of diet and chronological order of measurement

² Serum total cholesterol

³ Low density lipoprotein cholesterol

⁴ High density lipoprotein cholesterol

⁵ Triacylglycerols

^a Means differ between chickpea- and wheat-based diets (Repeated measures Analysis of variance, p<0.05)

Analysis revealed a substantial effect of the chickpea diet as a whole on the serum TC ($p=0.001$) and LDL-C ($p=0.002$) compared to the wheat diet. The apparent association between individual nutrients and lipids was assessed by univariate regression. Dietary fibre showed the strongest association, with a reduction in serum TC of 0.24 mmol/l (95% C.I. -0.47 to -0.02 mmol/l; $p=0.03$) and in serum LDL-C of 0.21 mmol/l (95% C.I. -0.42 to -0.01 mmol/l; $p=0.04$) for each increase in standard deviation in fibre intake. Multivariate analyses showed that about 55% of the difference in serum TC and 78% in LDL-C could be attributable to the combined effect of fibre and PUFA in the chickpea diet (Table 3), as these were the reductions in measured diet effect size when adjusted for these confounding variables.

Table 3 The combined effects of chickpea-supplemented versus wheat-supplemented diets and selected dietary components on total and LDL cholesterol¹

		Total Cholesterol			LDL		
		Effect size ²	95% CI	P value	Effect size ²	95% CI ²	P value
Diet:	Chickpea versus wheat	-0.10	(-0.31 to 0.11)	0.34	-0.04	(-.24 to 0.16)	0.72
Total fat:	(Mean 32.6 ± sd 6.5) % E ⁴	0.00	(-0.19 to 0.19)	0.99	0.15	(-0.04 to 0.33)	0.12
PUFA:	(Mean 16.5 ± sd 4.9) % TF ⁵	-0.12	(-0.35 to 0.11)	0.33	-0.19	(-0.33 to 0.07)	0.21
MUFA:	(Mean 38.9 ± sd 8.0) % TF ⁵	0.06	(-0.14 to 0.25)	0.56	-0.09	-0.25 to 0.07	0.26
Dietary fibre:	(Mean 29.2 ± sd 8.9) g	-0.24	(-0.48 to -0.0)	0.05	-0.24	-0.47 to 0.00	0.05

¹ Multivariate repeated measures ANOVA using general linear modelling. Each model includes the variables with recorded coefficients, adjusted for order of diet and chronological order of measurement, n=47

² Values expressed as mmol/L

³ Effect size for Diet is the difference in serum TC and LDL (mmol/L) between the two intervention diets. For the dietary components it is the effect of an increase of 1 std. dev. in the covariant on the serum total cholesterol and LDL (mmol/L)

⁴ Value expressed as percentage of daily energy intake

⁵ Value expressed as percentage of daily total fat intake

Discussion

This study investigated and compared a chickpea-supplemented and a wheat-supplemented diet (of at least five weeks of dietary intervention) in 47 men and women attending two separate centres, and showed a significantly lower concentration of serum TC and LDL-C after the chickpea-supplemented diet.

Although a small but significant difference was observed in the protein, carbohydrate and MUFA intake between the two intervention diets, statistical analyses indicated that this was not a significant effect. Most of the differences in the lipids and lipoproteins could be attributed the chickpea diet as a whole, and the small differences in the dietary fibre and PUFA on the chickpea diet compared to the wheat diet. Interpretation of this should be cautious, since dietary fibre and PUFA are characteristic components of the chickpeas, and regression analysis may be unreliable when separate measures of the same property (in this case dietary fibre and the PUFA on the one hand, and chickpea diet on the other) are included in the same regression model. Certainty about whether it is the fibre and PUFA components of chickpea having the effect as opposed to some other property of the chickpeas would require an experimental rather than a statistical approach.

A wealth of data is available from other dietary intervention trials showing much larger reductions in serum total and LDL cholesterol with increased intake of fibre or PUFA. However, most studies have included substantially higher amounts of fibre or PUFA to show those changes. Cholesterol-lowering by high

fibre diets is best observed in studies where the dietary fibre intake is very high (25), as much as two to three times the recommended intake (138). Similarly, although high PUFA diets have shown the changes in serum lipids, concerns have been raised about the effects of high intake of PUFA (>8% of total energy) reducing the HDL-C concentrations (44). In contrast, we present here a palatable food (chickpea) containing slightly higher amounts of dietary fibre and PUFA compared to wheat, that can be easily substituted for small amounts of wheat in weight maintenance 'Western' diet to show a small but significant change in serum TC and LDL-C without affecting the HDL-C.

Earlier studies that investigated the hypocholesterolemic effects of chickpeas (variety used was Bengal Gram) reported greater reductions in serum cholesterol concentrations than were observed in the present investigation (49, 106). This may have been due to the differences in the population studied, the study design and/or the type of chickpeas used. While the earlier studies were in Indians, the present study group was mainly Caucasian. The chickpeas more commonly consumed in India and Pakistan (desi/Bengal gram) contains about three times more fibre than the Kabuli chickpeas used in our study (79). In addition, the previous research protocols first induced hypercholesterolemia (with high fat diets of 10 weeks) before adding chickpeas to the diet (106).

Thus, chickpeas may have a role in reducing CHD risk. Although the differences in serum total and LDL-C were small (approximately 4%) previous research suggests that a 5% reduction ($\sim 0.3\text{mmol/L}$) in total cholesterol through dietary

intervention may reduce the risk of ischemic heart disease by about 15% at the age of 60 years (179). The results from the present investigation would equate to a 13.5% reduced risk. They were achieved with a practical dietary fibre intake around the recommended dietary intake of 30g/day (37). Further research is required to evaluate whether the differences observed in the present controlled situation prevail or are accentuated when chickpeas are added to the long term *ad libitum* diet.

Acknowledgements

We would like to thank the participants of the study. The Grain Research Development Corporation (Canberra, ACT, Australia) provided the funds and the Clifford Craig Medical Research Trust (Launceston, Tasmania) provided the clinical room facilities.

Appendix 2

Abstracts and Posters from Conference Presentations

The effect of chickpeas on human serum lipids and lipoproteins

JK Pittaway¹, KDK Ahuja¹, A Chronopoulos², M. Cehun², IK Robertson¹, PJ Nestel²,
MJ Ball¹,

¹*School of Human Life Sciences, University of Tasmania, Launceston TAS 7250*

²*Baker Heart Research Institute, Melbourne VIC 8008*

Background- Consumption of pulses has been associated with reduction of hypercholesterolaemia and reduced risk of coronary heart disease (CHD). Chickpeas have been a staple part of Indian, Mediterranean and African diets for many thousands of years but are a relatively novel addition to Western cuisine.

Objective- To compare the effect of a chickpea-supplemented diet with a wheat-based diet on human serum lipids and lipoproteins.

Design- Randomized, crossover dietary interventions each at least five weeks in duration, involving 47 free-living adults with at least one CHD risk factor, or a family history of CHD. Intervention diets were isoenergetic to the participants' usual diet, designed to be matched for macronutrient content and controlled for dietary fibre. Chickpeas were consumed in the form of canned, drained chickpeas and in bread and biscuits containing 30% chickpea flour. Results were analysed using repeated measures ANOVA by general linear modelling.

Outcomes- Reductions in the concentration of serum total cholesterol (3.9%) and low density lipoprotein-cholesterol (4.7%) on completion of the chickpea diet compared to the wheat diet. When corrected for the effect of gender, age, total fat, percent fatty acid composition and dietary fibre, the effect of diet on total cholesterol and low density lipoprotein cholesterol disappeared.

Conclusions- Despite attempts at controlling macronutrient intake, the inclusion of chickpeas in the intervention diet caused changes in dietary fat and fibre composition, leading to reduced serum total and low density lipoprotein cholesterol.

Sponsorship- Grain Research Development Corporation, Australia

The Effect of Chickpeas on Serum Lipids

JK Pittaway¹, KDK Ahuja¹, M Cehun², A Chronopoulos², IK Robertson¹, PJ Nestel², MJ Ball¹

¹School of Human Life Sciences, University of Tasmania, TAS 7250

²Baker Heart Research Institute, Melbourne VIC 8008

Background: Consumption of pulses has been associated with reduction of hypercholesterolaemia and reduced risk of coronary heart disease (CHD). Chickpeas have been a staple part of Indian, Mediterranean and African diets for many thousands of years but are a relatively novel addition to Western cuisine.

Objective: To compare the effect of a chickpea-supplemented diet with a wheat-based diet on human serum lipids and lipoproteins.

Design: Randomised, crossover dietary interventions each at least five weeks in duration, involving 47 free-living adults (28 women, 19 men) with at least one heart disease risk factor (age >40 years, obesity, hypertension, sedentary lifestyle, elevated blood cholesterol) or a family history of CHD. Intervention diets were isoenergetic to the participants' usual diet, designed to be matched for macronutrient content and controlled for dietary fibre. Chickpeas were consumed in the form of canned drained chickpeas and in bread and biscuits containing 30% chickpea flour. A wheat-based diet was used as the comparison because the 'Australian' diet is mostly wheat-based. Results were analysed using repeated measures ANOVA by general linear modelling.

Results: The mean (SD) age of the study group was 53.9 (9.8) years and BMI 27.6 (4.1). There was no change in body weight after the two dietary periods. The two diets were not significantly different in energy content but differed in dietary fatty acid composition, carbohydrate and dietary fibre (see Table 1).

Table 1. Daily macronutrient intake on intervention diets.

Macronutrient	Unit	Diet (mean ± SD) n=43	
		Chickpea	Wheat
Total energy	MJ	8.3 ± 2.3	8.5 ± 3.0
Protein	%E	18.1 ± 2.7	19.0 ± 2.9
Carbohydrate	%E	45.8 ± 6.4 *	44.1 ± 6.1
Total fat	%E	32.0 ± 6.6	33.1 ± 6.4
- Saturated	%TF	38.0 ± 7.8	39.2 ± 6.7
- Polyunsaturated	%TF	17.1 ± 5.2	15.8 ± 4.7
- Monounsaturated	%TF	37.3 ± 8.5 *	40.6 ± 7.1
Dietary fibre	g	30.5 ± 7.0 *	27.9 ± 10.4

%E percent energy; %TF percent total fat;

* significant difference (Repeated measures ANOVA, p<0.05)

This study was funded by the Grain Research Development Corporation, Australia. Research space was kindly provided by the Clifford Craig Medical Research Tasmania.

A significantly lower concentration of serum total cholesterol (3.9%) and low density lipoprotein cholesterol (4.7%) was found on completion of the chickpea diet compared to the wheat diet. No difference was detected in serum high density lipoprotein cholesterol or triglycerides.

Table 2. Lipid profiles of participants after each diet.

Analyte	Unit	Diet (mean ± SD), n=47	
		Chickpea	Wheat
TC	mmol/L	5.68 ± 0.97*	5.91 ± 0.95
LDL-C	mmol/L	3.69 ± 0.81*	3.87 ± 0.84
HDL-C	mmol/L	1.34 ± 0.32	1.37 ± 0.32
Triglycerides	mmol/L	1.43 ± 0.83	1.48 ± 0.66

TC serum total cholesterol; LDL-C serum low density lipoprotein-cholesterol

HDL-C serum high density lipoprotein-cholesterol

* significant difference (Repeated measures ANOVA, p<0.05)

When corrected for the effect of gender, age, total fat, percent fatty acid composition and dietary fibre, the effect of the chickpea diet on serum total cholesterol was reduced from a difference of 0.22 mmol/L (95% C.I. 0.10 to 0.35 mmol/L, p=0.001) to a difference of 0.10 mmol/L (95% C.I. -0.31 to 0.11 mmol/L, p=0.34). The corrected effect of the chickpea diet on low density lipoprotein-cholesterol was reduced from a difference of 0.18 mmol/L (95% C.I. 0.07 to 0.29 mmol/L, p=0.002) to a difference of 0.04 mmol/L (95% C.I. -0.24 to 0.16 mmol/L, p=0.72).

Conclusion: Including chickpeas in an Australian diet improved the lipid profile consistent with reduced risk of CHD. It was not possible to determine whether this was due to the changes in dietary fat and fibre, or the specific presence of chickpeas – although this distinction may be of little practical importance. Further investigation is warranted.



Effects of a controlled diet supplemented with chickpeas versus wheat on serum lipids, glycaemic control, satiety and bowel function

JK Pittaway, KDK Ahuja, IK Robertson, MJ Ball

School of Human Life Sciences University of Tasmania, Launceston, TAS 7250

Background - Chickpeas are common in many ethnic diets and are rich in polyunsaturated fatty acids (PUFA), dietary fibre and resistant starch. However, little information is available on the health effects of regular chickpea consumption.

Objective - To compare the effects of a diet supplemented with chickpeas to a wheat-supplemented diet of similar fibre content on serum lipids and glycaemic control, and to compare these diets plus a wheat based diet of low fibre content on satiety and bowel function.

Design - Twenty-seven free-living adults followed two randomized, crossover dietary interventions each of five weeks duration. The chickpea diet included canned chickpeas (140g/day), bread and biscuits containing 30% chickpea flour. The diets were isoenergetic to the participants' usual diet, matched for macronutrient content and controlled for dietary fibre.

Following on from the second randomised intervention, a sub-group of 18 participants underwent a third lower-fibre wheat diet. Measures at the end of the diets were compared by repeated measures ANOVA using GLM.

Outcomes - Serum TC was 0.25 mmol/L ($p < 0.01$) and LDL-C was 0.20 mmol/L lower ($p = 0.02$) following the chickpea diet compared to the wheat diet. An unintended significant increase in PUFA and corresponding decrease in MUFA consumption occurred during the chickpea diet and statistical adjustment for this reduced the effect on serum lipids by about 50%. There was no significant difference in glucose or insulin concentrations. Perceived general bowel health improved significantly during the chickpea diet although there was considerable individual variation. Greater satiety was reported by some participants and was significantly greater than on the low fibre diet.

Conclusions - The small but significantly lower serum TC and LDL-C during the chickpea diet could provide a valuable health benefit.

Chickpeas influence P:S ratio and fibre content of *ad libitum* dietary intake leading to improved serum lipid profile, glycaemic control and satiation

JK Pittaway, IK Robertson, MJ Ball

School of Human Life Sciences, University of Tasmania, Launceston Tas 7250

Background - There has been a gradual development of interest in the contribution of pulses to a healthy lifestyle, as awareness of ethnic diets and lifestyles has grown. Controlled dietary intervention studies with chickpeas have shown a small but significant reduction in serum low density lipoprotein (LDL-C) and total cholesterol (TC) concentrations in women and men. What would be the effect of chickpeas on nutrient intake, metabolic and physiological changes in a more realistic *ad libitum* setting?

Objective - To estimate the effect of including a realistic quantity of chickpeas in an otherwise *ad libitum* diet of free-living adults.

Design - Ordered crossover design of 20 weeks duration. Forty-five adult women and men, as a group slightly hypercholesterolaemic but normoglycaemic, included 104g of chickpeas per day in their habitual diet for 12 weeks.

Comparison of macronutrient and dietary fibre consumption, body weight, body mass index, fasting plasma glucose, serum lipids, lipoproteins, insulin, leptin and ghrelin concentrations, after habitual diet supplemented with chickpeas and after four weeks of post chickpea *ad libitum* diet. Semi-quantitative assessment of bowel function and satiation using anchored visual analogue scales. All data was analysed with repeated measures ANOVA using GLM with robust standard error estimation and ordinal logistic regression for ordinal data.

Outcomes - Chickpea-related increases in mean dietary fibre and PUFA intake were associated with significant decreases in serum TC and LDL-C, fasting insulin and HOMA-IR ($p < 0.05$ for all) when compared to the usual dietary phase. Small but significant reductions in body weight ($p = 0.001$) with increased perceived satiation and improved bowel function were noted during the chickpea phase compared to the usual dietary phase.

Conclusion - Adding chickpeas to the diet is an option for individuals wanting to modify their diet-associated CVD risk factors.

References

1. AIHW Heart, stroke and vascular diseases - Australian facts 2001. AIHW Cat. No. CVD 13. Canberra, Australia: Australian Institute of Health and Welfare, National Heart Foundation of Australia, National Stroke Foundation of Australia; 2001.
2. AIHW Heart, stroke and vascular diseases - Australian facts 2004. AIHW Cat. No. CVD 27. Canberra, Australia: Australian Institute of Health and Welfare and National Heart Foundation of Australia; 2004.
3. Al-Hasani, H., and Joost, H. G. Nutrition-/diet-induced changes in gene expression in white adipose tissue. *Best Pract Res Clin Endocrinol Metab* 19:589-603; 2005.
4. Almario, R. U., Vonghavaravat, V., Wong, R., and Kasim-Karakas, S. E. Effects of walnut consumption on plasma fatty acids and lipoproteins in combined hyperlipidemia. *Am J Clin Nutr* 74:72-9; 2001.
5. Anderson, J. W. Dietary fibre, complex carbohydrate and coronary artery disease. *Can J Cardiol* 11 Suppl G:55G-62G; 1995.
6. Anderson, J. W., Chen, W. J., and Sieling, B. Hypolipidemic effects of high-carbohydrate, high-fiber diets. *Metabolism* 29:551-8; 1980.
7. Anderson, J. W., and Hanna, T. J. Impact of nondigestible carbohydrates on serum lipoproteins and risk for cardiovascular disease. *J Nutr* 129:1457S-66S; 1999.
8. Anderson, J. W., Johnstone, B. M., and Cook-Newell, M. E. Meta-analysis of the effects of soy protein intake on serum lipids. *N Engl J Med* 333:276-82; 1995.
9. Anderson, J. W., and Major, A. W. Pulses and lipaemia, short- and long-term effect: potential in the prevention of cardiovascular disease. *Br J Nutr* 88 Suppl 3:S263-71; 2002.
10. Anderson, J. W., Story, L., Sieling, B., Chen, W. J., Petro, M. S., and Story, J. Hypocholesterolemic effects of oat-bran or bean intake for hypercholesterolemic men. *Am J Clin Nutr* 40:1146-55; 1984.

11. Anderson, J. W., Zeigler, J. A., Deakins, D. A., Floore, T. L., Dillon, D. W., Wood, C. L., Oeltgen, P. R., and Whitley, R. J. Metabolic effects of high-carbohydrate, high-fiber diets for insulin-dependent diabetic individuals. *Am J Clin Nutr* 54:936-43; 1991.
12. Ascherio, A., and Willett, W. C. New directions in dietary studies of coronary heart disease. *J Nutr* 125:647S-655S; 1995.
13. Ashton, E., and Ball, M. Effects of soy as tofu vs meat on lipoprotein concentrations. *Eur J Clin Nutr* 54:14-9; 2000.
14. Astrup, A., Grunwald, G. K., Melanson, E. L., Saris, W. H., and Hill, J. O. The role of low-fat diets in body weight control: a meta-analysis of ad libitum dietary intervention studies. *Int J Obes Relat Metab Disord* 24:1545-52; 2000.
15. Barclay, A. W., Brand-Miller, J. C., and Wolever, T. M. Glycemic index, glycemic load, and glycemic response are not the same. *Diabetes Care* 28:1839-40; 2005.
16. Barringer, T. A. Mediterranean diets and cardiovascular disease. *Curr Atheroscler Rep* 3:437-45; 2001.
17. Bazzano, L. A., He, J., Ogden, L. G., Loria, C., Vupputuri, S., Myers, L., and Whelton, P. K. Legume consumption and risk of coronary heart disease in US men and women: NHANES I Epidemiologic Follow-up Study. *Arch Intern Med* 161:2573-8; 2001.
18. Birender, K., Soni, G. L., and Singh, R. Nutritional evaluation of gram (*Cicer arietinum*) varieties. *Hum Nutr Food Sci Nutr* 41F:121-128; 1987.
19. Blackwood, A. D., Salter, J., Dettmar, P. W., and Chaplin, M. F. Dietary fibre, physicochemical properties and their relationship to health. *J R Soc Health* 120:242-7.; 2000.
20. Bray, G. A. Risks of obesity. *Endocrinol Metab Clin North Am* 32:787-804, viii; 2003.
21. Bray, G. A., and Popkin, B. M. Dietary fat intake does affect obesity! *Am J Clin Nutr* 68:1157-73; 1998.

22. Brown, L., Rosner, B., Willett, W. W., and Sacks, F. M. Cholesterol-lowering effects of dietary fiber: a meta-analysis. *Am J Clin Nutr* 69:30-42; 1999.
23. Burkitt, D. P., Walker, A. R., and Painter, N. S. Dietary fiber and disease. *JAMA* 229:1068-74; 1974.
24. Burley, V. J., Paul, A. W., and Blundell, J. E. Influence of a high-fibre food (myco-protein) on appetite: effects on satiation (within meals) and satiety (following meals). *Eur J Clin Nutr* 47:409-18.; 1993.
25. Champ, M., Langkilde, A. M., Brouns, F., Kettlitz, B., and Collet, Y. L. Advances in dietary fibre characterisation. 1. Definition of dietary fibre, physiological relevance, health benefits and analytical aspects. *Nutrition Research Reviews* 16:71-82; 2003.
26. Chandalia, M., Garg, A., Lutjohann, D., von Bergmann, K., Grundy, S. M., and Brinkley, L. J. Beneficial effects of high dietary fiber intake in patients with type 2 diabetes mellitus. *N Engl J Med* 342:1392-8; 2000.
27. Clarke, R., Frost, C., Collins, R., Appleby, P., and Peto, R. Dietary lipids and blood cholesterol: quantitative meta-analysis of metabolic ward studies. *BMJ* 314:112-7; 1997.
28. Cummings, J. H., Antoine, J. M., Azpiroz, F., Bourdet-Sicard, R., Brandtzaeg, P., Calder, P. C., Gibson, G. R., Guarner, F., Isolauri, E., Pannemans, D., Shortt, C., Tuijtelaars, S., and Watzl, B. PASSCLAIM--gut health and immunity. *Eur J Nutr* 43 Suppl 2:II118-II173; 2004.
29. Dahl, W. J., Whiting, S. J., Healey, A., Zello, G. A., and Hildebrandt, S. L. Increased stool frequency occurs when finely processed pea hull fiber is added to usual foods consumed by elderly residents in long-term care. *J Am Diet Assoc* 103:1199-202; 2003.
30. Darmadi-Blackberry, I., Wahlqvist, M. L., Kouris-Blazos, A., Steen, B., Lukito, W., Horie, Y., and Horie, K. Legumes: the most important dietary predictor of survival in older people of different ethnicities. *Asia Pac J Clin Nutr* 13:217-20; 2004.

31. de Graaf, C., Blom, W. A., Smeets, P. A., Stafleu, A., and Hendriks, H. F. Biomarkers of satiation and satiety. *Am J Clin Nutr* 79:946-61; 2004.
32. de Jong, A., Plat, J., and Mensink, R. P. Metabolic effects of plant sterols and stanols (Review). *J Nutr Biochem* 14:362-9; 2003.
33. de Lorgeril, M., Salen, P., Martin, J. L., Monjaud, I., Delaye, J., and Mamelle, N. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. *Circulation* 99:779-85; 1999.
34. de Lorgeril, M., Salen, P., Monjaud, I., and Delaye, J. The 'diet heart' hypothesis in secondary prevention of coronary heart disease. *Eur Heart J* 18:13-8; 1997.
35. Denke, M. A. Review of human studies evaluating individual dietary responsiveness in patients with hypercholesterolemia. *Am J Clin Nutr* 62:471S-477S; 1995.
36. Deshpande, S. S. Food legumes in human nutrition: A personal perspective. *CRC Food Sci Nutr* 32:333-363; 1992.
37. DHFS The Australian Guide to Healthy Eating. Canberra, Australia: Department of Health and Family Services - Australian Government; 1998.
38. Dilawari, J. B., Kumar, V. K., Khurana, S., Bhatnagar, R., and Dash, R. J. Effect of legumes on blood sugar in diabetes mellitus. *Indian J Med Res* 85:184-7; 1987.
39. DSLabs Insulin RIA Package Insert. pp. 1-7. Oxon, UK: Diagnostic Systems Laboratories; 2003.
40. Duane, W. C. Effects of legume consumption on serum cholesterol, biliary lipids, and sterol metabolism in humans. *J Lipid Res* 38:1120-8; 1997.
41. Edington, J., Thorogood, M., Geekie, M., Ball, M., and Mann, J. Assessment of nutritional intake using dietary records with estimated weights. *J Hum Nutr Diet* 2:407-414; 1989.

42. Erdman, J. W., Jr. AHA Science Advisory: Soy protein and cardiovascular disease: A statement for healthcare professionals from the Nutrition Committee of the AHA. *Circulation* 102:2555-9; 2000.
43. Fehily, A. Legumes: types and nutritional value. In: M. Sadler (ed.), *Encyclopedia of Human Nutrition* 2, pp. 1181-1188. New York: Academic Press; 1999.
44. Foley, M., Ball, M., Chisholm, A., Duncan, A., Spears, G., and Mann, J. Should Monounsaturated or Polyunsaturated Fats Replace Saturated Fat in the Diet. *European Journal of Clinical Nutrition* 46:429-436; 1992.
45. Friedewald, W., Levy, R., and Fredrickson, D. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* 18:499-502; 1972.
46. Fukagawa, N., Anderson, J., Hageman, G., Young, V., and Minaker, K. High-carbohydrate, high-fiber diets increase peripheral insulin sensitivity in healthy young and old adults. *Am J Clin Nutr* 52:524-528; 1990.
47. Galvin, M. A., Kiely, M., Harrington, K. E., Robson, P. J., Moore, R., and Flynn, A. The North/South Ireland Food Consumption Survey: the dietary fibre intake of Irish adults. *Public Health Nutr* 4:1061-8.; 2001.
48. Geil, P. B., and Anderson, J. W. Nutrition and health implications of dry beans: a review. *J Am Coll Nutr* 13:549-58; 1994.
49. Ghorai, M., Mandal, S. C., Pal, M., Pal, S. P., and Saha, B. P. A comparative study on hypocholesterolaemic effect of allicin, whole germinated seeds of bengal gram and guggulipid of gum gugglu. *Phytother Res* 14:200-2.; 2000.
50. Glore, S. R., Van Treeck, D., Knehans, A. W., and Guild, M. Soluble fiber and serum lipids: a literature review. *J Am Diet Assoc* 94:425-36; 1994.
51. GRDC Dietary Fibre and Resistant Starch. 2002: <http://www.grdc.com.au>; 2002.
52. Grundy, S. M., and Denke, M. A. Dietary influences on serum lipids and lipoproteins. *J Lipid Res* 31:1149-72; 1990.

53. Guillon, F., and Champ, M. M. Carbohydrate fractions of legumes: uses in human nutrition and potential for health. *Br J Nutr* 88 Suppl 3:S293-306; 2002.
54. Haas, V., Onur, S., Paul, T., Nutzinger, D. O., Bosy-Westphal, A., Hauer, M., Brabant, G., Klein, H., and Muller, M. J. Leptin and body weight regulation in patients with anorexia nervosa before and during weight recovery. *Am J Clin Nutr* 81:889-96; 2005.
55. Haslam, D. W., and James, W. P. Obesity. *Lancet* 366:1197-209; 2005.
56. Havel, P. J. Control of energy homeostasis and insulin action by adipocyte hormones: leptin, acylation stimulating protein, and adiponectin. *Curr Opin Lipidol* 13:51-9; 2002.
57. Hegsted, D. M., McGandy, R. B., Myers, M. L., and Stare, F. J. Quantitative effects of dietary fat on serum cholesterol in man. *Am J Clin Nutr* 17:281-95; 1965.
58. Heilbronn, L. K., Noakes, M., and Clifton, P. M. Effect of energy restriction, weight loss, and diet composition on plasma lipids and glucose in patients with type 2 diabetes. *Diabetes Care* 22:889-95; 1999.
59. Hensrud, D. D. Dietary treatment and long-term weight loss and maintenance in type 2 diabetes. *Obes Res* 9 Suppl 4:348S-353S; 2001.
60. Hermans, M. P., Levy, J. C., Morris, R. J., and Turner, R. C. Comparison of insulin sensitivity tests across a range of glucose tolerance from normal to diabetes. *Diabetologia* 42:678-87; 1999.
61. Holt, S. H., Delargy, H. J., Lawton, C. L., and Blundell, J. E. The effects of high-carbohydrate vs high-fat breakfasts on feelings of fullness and alertness, and subsequent food intake. *Int J Food Sci Nutr* 50:13-28; 1999.
62. Holt, S. H., Miller, J. C., Petocz, P., and Farmakalidis, E. A satiety index of common foods. *Eur J Clin Nutr* 49:675-90; 1995.
63. Howarth, N. C., Saltzman, E., and Roberts, S. B. Dietary fiber and weight regulation. *Nutr Rev* 59:129-39; 2001.

64. Howell, W. H., McNamara, D. J., Tosca, M. A., Smith, B. T., and Gaines, J. A. Plasma lipid and lipoprotein responses to dietary fat and cholesterol: a meta-analysis. *Am J Clin Nutr* 65:1747-64; 1997.
65. Hu, F. B., Manson, J. E., and Willett, W. C. Types of dietary fat and risk of coronary heart disease: a critical review. *J Am Coll Nutr* 20:5-19; 2001.
66. Hu, F. B., and Willett, W. C. Optimal diets for prevention of coronary heart disease. *JAMA* 288:2569-78; 2002.
67. Hung, T., Sievenpiper, J. L., Marchie, A., Kendall, C. W., and Jenkins, D. J. Fat versus carbohydrate in insulin resistance, obesity, diabetes and cardiovascular disease. *Curr Opin Clin Nutr Metab Care* 6:165-76; 2003.
68. iGourmet.com Garbanzos. pp. 1: <http://www.igourmet.com>; 2002.
69. James, S. L., Muir, J. G., Curtis, S. L., and Gibson, P. R. Dietary fibre: a roughage guide. *Intern Med J* 33:291-6; 2003.
70. James, W. P. T. Policy and a Prudent Diet. In: J. S. Garrow, W. P. T. James, and A. Ralph (eds.), *Human Nutrition and Dietetics*, pp. 837-845. London, UK: Churchill-Livingstone; 2000.
71. Jenkins, D. J., Kendall, C. W., Augustin, L. S., and Vuksan, V. High-complex carbohydrate or lente carbohydrate foods? *Am J Med* 113 Suppl 9B:30S-37S; 2002.
72. Jenkins, D. J., Kendall, C. W., Popovich, D. G., Vidgen, E., Mehling, C. C., Vuksan, V., Ransom, T. P., Rao, A. V., Rosenberg-Zand, R., Tariq, N., Corey, P., Jones, P. J., Raeini, M., Story, J. A., Furumoto, E. J., Illingworth, D. R., Pappu, A. S., and Connelly, P. W. Effect of a very-high-fiber vegetable, fruit, and nut diet on serum lipids and colonic function. *Metabolism* 50:494-503; 2001.
73. Jenkins, D. J., Wolever, T. M., Jenkins, A. L., Thorne, M. J., Lee, R., Kalmusky, J., Reichert, R., and Wong, G. S. The glycaemic index of foods tested in diabetic patients: a new basis for carbohydrate exchange favouring the use of legumes. *Diabetologia* 24:257-64; 1983.

74. Jenkins, D. J., Wolever, T. M., Taylor, R. H., Barker, H. M., and Fielden, H. Exceptionally low blood glucose response to dried beans: comparison with other carbohydrate foods. *Br Med J* 281:578-80; 1980.
75. Jenkins, D. J., Wong, G. S., Patten, R., Bird, J., Hall, M., Buckley, G. C., McGuire, V., Reichert, R., and Little, J. A. Leguminous seeds in the dietary management of hyperlipidemia. *Am J Clin Nutr* 38:567-73; 1983.
76. Kalra, S. P., Ueno, N., and Kalra, P. S. Stimulation of appetite by ghrelin is regulated by leptin restraint: peripheral and central sites of action. *J Nutr* 135:1331-5; 2005.
77. Keys, A. Coronary Heart Disease in Seven Countries. *Circulation* 41:162-83; 1970.
78. Keys, A., Anderson, J. T., and Grande, F. Prediction of serum-cholesterol responses of man to changes in fats in the diet. *Lancet* 273:959-66; 1957.
79. Khan, M. A., Akhtar, N., Ullah, I., and Jaffery, S. Nutritional evaluation of desi and kabuli chickpeas and their products commonly consumed in Pakistan. *Int J Food Sci Nutr* 46:215-23; 1995.
80. Kim, W. W., Kelsay, J. L., Judd, J. T., Marshall, M. W., Mertz, W., and Prather, E. S. Evaluation of long-term dietary intakes of adults consuming self-selected diets. *Am J Clin Nutr* 40:1327-32; 1984.
81. Kingman, S. M., Walker, A. F., Low, A. G., Sambrook, I. E., Owen, R. W., and Cole, T. J. Comparative effects of four legume species on plasma lipids and faecal steroid excretion in hypercholesterolaemic pigs. *Br J Nutr* 69:409-21; 1993.
82. Klein, S. Clinical trial experience with fat-restricted vs. carbohydrate-restricted weight-loss diets. *Obes Res* 12 Suppl 2:141S-4S; 2004.
83. Kojima, M., and Kangawa, K. Ghrelin: structure and function. *Physiol Rev* 85:495-522; 2005.
84. Kopelman, P. G. Obesity as a medical problem. *Nature* 404:635-43; 2000.
85. Kouris-Blazos, A., Gnardellis, C., Wahlqvist, M. L., Trichopoulos, D., Lukito, W., and Trichopoulou, A. Are the advantages of the

- Mediterranean diet transferable to other populations? A cohort study in Melbourne, Australia. *Br J Nutr* 82:57-61; 1999.
86. Kris-Etherton, P. M., and Yu, S. Individual fatty acid effects on plasma lipids and lipoproteins: human studies. *Am J Clin Nutr* 65:1628S-1644S; 1997.
 87. Kritchevsky, D. Dietary protein, cholesterol and atherosclerosis: A review of the early history. *J Nutr* 125:589S-593S; 1995.
 88. Kushi, L. H., Lenart, E. B., and Willett, W. C. Health implications of Mediterranean diets in light of contemporary knowledge. 1. Plant foods and dairy products. *Am J Clin Nutr* 61:1407S-1415S; 1995.
 89. Kushi, L. H., Lew, R. A., Stare, F. J., Ellison, C. R., el Lozy, M., Bourke, G., Daly, L., Graham, I., Hickey, N., Mulcahy, R., and et al. Diet and 20-year mortality from coronary heart disease. The Ireland-Boston Diet-Heart Study. *N Engl J Med* 312:811-8; 1985.
 90. Kushi, L. H., Meyer, K. A., and Jacobs, D. R., Jr. Cereals, legumes, and chronic disease risk reduction: evidence from epidemiologic studies. *Am J Clin Nutr* 70:451S-458S; 1999.
 91. Lavigne, C., Marette, A., and Jacques, H. Cod and soy proteins compared with casein improve glucose tolerance and insulin sensitivity in rats. *Am J Physiol Endocrinol Metab* 278:E491-500; 2000.
 92. Lewis, S. J., and Heaton, K. W. The intestinal effects of bran-like plastic particles: is the concept of 'roughage' valid after all? *Eur J Gastroenterol Hepatol* 9:553-7.; 1997.
 93. Lewis, S. J., and Heaton, K. W. Stool form scale as a useful guide to intestinal transit time. *Scand J Gastroenterol* 32:920-4.; 1997.
 94. Li, B. W., Andrews, K. W., and Pehrsson, P. R. Individual sugars, soluble, and insoluble dietary fiber contents of 70 high consumption foods. *J Food Compos Anal* 15:715-723; 2002.
 95. Lo, G. S., Goldberg, A. P., Lim, A., Grundhauser, J. J., Anderson, C., and Schonfeld, G. Soy fiber improves lipid and carbohydrate metabolism in primary hyperlipidemic subjects. *Atherosclerosis* 62:239-48; 1986.

96. Louis, T. A., Lavori, P. W., Bailar, J. C., 3rd, and Polansky, M. Crossover and self-controlled designs in clinical research. *N Engl J Med* 310:24-31; 1984.
97. Ludwig, D. S. The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. *JAMA* 287:2414-23; 2002.
98. Macfarlane, G. T., and Cummings, J. H. The colonic flora, fermentation and large bowel digestive function. In: H. F. Phillips, H. F. Pemberton, and H. F. Shorter (eds.), *The large intestine: physiology, pathology and diseases*, pp. 51-92. New York: Raven Press; 1991.
99. Mackay, S., and Ball, M. J. Do beans and oat bran add to the effectiveness of a low-fat diet? *Eur J Clin Nutr* 46:641-648; 1992.
100. Mahalko, J. R., Sandstead, H. H., Johnson, L. K., Inman, L. F., Milne, D. B., Warner, R. C., and Haunz, E. A. Effect of consuming fiber from corn bran, soy hulls, or apple powder on glucose tolerance and plasma lipids in type II diabetes. *Am J Clin Nutr* 39:25-34; 1984.
101. Mani, U. V., Bhatt, S., Mehta, N. C., Pradhan, S. N., Shah, V., and Mani, I. Glycemic index of traditional Indian carbohydrate foods. *J Am Coll Nutr* 9:573-7; 1990.
102. Markovic, T. P., Jenkins, A. B., Campbell, L. V., Furler, S. M., Kraegen, E. W., and Chisholm, D. J. The determinants of glycemic responses to diet restriction and weight loss in obesity and NIDDM. *Diabetes Care* 21:687-94; 1998.
103. Marlett, J. A., McBurney, M. I., and Slavin, J. L. Position of the American Dietetic Association: health implications of dietary fiber. *J Am Diet Assoc* 102:993-1000; 2002.
104. Marmot, M. Coronary Heart Disease: rise and fall of a modern epidemic. In: M. E. Marmot, P. (ed.), *Coronary Heart Disease: from aetiology to public health*, pp. 3 -19. Oxford: Oxford Medical Publications; 1992.
105. Mathur, K. S. Hypocholesterolaemic Action of Bengal Gram. Symposium on Nutrition And Heart Disease, pp. 19-33. New Delhi, India: ICMR; 1971.

106. Mathur, K. S., Khan, M. A., and Sharma, R. D. Hypocholesterolaemic effect of Bengal gram: a long-term study in man. *Br Med J* 1:30-1; 1968.
107. Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., and Turner, R. C. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412-9.; 1985.
108. Mazur, W. M., Duke, J. A., Wahala, K., Rasku, S., and Adlercreutz, H. Isoflavonoids and lignans in legumes: nutritional and health aspects in humans. *J Nutr Biochem* 9:193-200; 1998.
109. McKeown, N. M., Meigs, J. B., Liu, S., Saltzman, E., Wilson, P. W., and Jacques, P. F. Carbohydrate nutrition, insulin resistance, and the prevalence of the metabolic syndrome in the Framingham Offspring Cohort. *Diabetes Care* 27:538-46; 2004.
110. Menotti, A. Diet, cholesterol and coronary heart disease. A perspective. *Acta Cardiol* 54:169-72; 1999.
111. Menotti, A., Kromhout, D., Blackburn, H., Fidanza, F., Buzina, R., and Nissinen, A. Food intake patterns and 25-year mortality from coronary heart disease: cross-cultural correlations in the Seven Countries Study. The Seven Countries Study Research Group. *Eur J Epidemiol* 15:507-15; 1999.
112. Mensink, R. P., Zock, P. L., Kester, A. D., and Katan, M. B. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am J Clin Nutr* 77:1146-55; 2003.
113. Mertz, W., Tsui, J. C., Judd, J. T., Reiser, S., Hallfrisch, J., Morris, E. R., Steele, P. D., and Lashley, E. What are people really eating? The relation between energy intake derived from estimated diet records and intake determined to maintain body weight. *Am J Clin Nutr* 54:291-5; 1991.
114. Messina, M. Soy, soy phytoestrogens (isoflavones), and breast cancer. *Am J Clin Nutr* 70:574-5; 1999.

115. Messina, M. J. Legumes and soybeans: overview of their nutritional profiles and health effects. *Am J Clin Nutr* 70:439S-450S; 1999.
116. Muir, J. G., and O'Dea, K. Measurement of resistant starch: factors affecting the amount of starch escaping digestion in vitro. *Am J Clin Nutr* 56:123-7; 1992.
117. Muir, J. G., Yeow, E. G., Keogh, J., Pizzey, C., Bird, A. R., Sharpe, K., O'Dea, K., and Macrae, F. A. Combining wheat bran with resistant starch has more beneficial effects on fecal indexes than does wheat bran alone. *Am J Clin Nutr* 79:1020-8; 2004.
118. Murkies, A. L., Wilcox, G., and Davis, S. R. Clinical review 92: Phytoestrogens. *J Clin Endocrinol Metab* 83:297-303; 1998.
119. Nagata, C., Takatsuka, N., Kurisu, Y., and Shimizu, H. Decreased serum total cholesterol concentration is associated with high intake of soy products in Japanese men and women. *J Nutr* 128:209-13; 1998.
120. Ness, A. R., and Powles, J. W. Fruit and vegetables, and cardiovascular disease: a review. *Int J Epidemiol* 26:1-13; 1997.
121. Nestel, P., Cehun, M., and Chronopoulos, A. Effects of long-term consumption and single meals of chickpeas on plasma glucose, insulin, and triacylglycerol concentrations. *Am J Clin Nutr* 79(3) , p. 390-5; 2004.
122. Newman, C. W., Newman, R. K., and Lockerman, R. H. Utilization of food legumes in human nutrition. In: R. J. Summerfield (ed.), *World Crops: Cool season food legumes*, pp. 405-11; 1988.
123. NHMRC/DCSH Recommended Dietary Intakes for Use in Australia. Canberra Australia: National Health and Medical Research Council, Department of Community Services and Health; 1991.
124. Nityanand, S., and Kapoor, N. K. Effect of Bengal gram, *Cicer arietinum* L, on experimental atherosclerosis. *Indian J Exp Biol* 11:65-6; 1973.
125. Noakes, M., Clifton, P., and McMurchie, T. The role of diet in cardiovascular health. A review of the evidence. *Aust J Nutr Diet* 56:S3-S22; 1999.

126. O'Dea, K. Factors Influencing Carbohydrate Digestion: Acute and long term consequences. *Diab Nutr Metab* 3:251-258; 1990.
127. O'Dea, K., Traianedes, K., Ireland, P., Niall, M., Sadler, J., Hopper, J., and De Luise, M. The effects of diet differing in fat, carbohydrate, and fiber on carbohydrate and lipid metabolism in type II diabetes. *J Am Diet Assoc* 89:1076-86; 1989.
128. Pereira, M. A., O'Reilly, E., Augustsson, K., Fraser, G. E., Goldbourt, U., Heitmann, B. L., Hallmans, G., Knekt, P., Liu, S., Pietinen, P., Spiegelman, D., Stevens, J., Virtamo, J., Willett, W. C., and Ascherio, A. Dietary fiber and risk of coronary heart disease: a pooled analysis of cohort studies. *Arch Intern Med* 164:370-6; 2004.
129. Petterson, D. S., Sipsas, S., and Mackintosh, J. B. Chickpea (*Cicer arietinum*). *The Chemical Composition and Nutritive Value of Australian Pulses*, pp. 13-14. Canberra, Australia: Grain Research and Development Corporation; 1997.
130. Phillips, R. D. Starchy legumes in human nutrition, health and culture. *Plant Food Hum Nutr* 44; 1993.
131. Pinto, R. J. Risk factors for coronary heart disease in Asian Indians: clinical implications for prevention of coronary heart disease. *Indian J Med Sci* 52:49-54; 1998.
132. Potter, S. M. Overview of proposed mechanisms for the hypocholesterolemic effect of soy. *J Nutr* 125:606S-611S; 1995.
133. Probert, C. J., Emmett, P. M., and Heaton, K. W. Intestinal transit time in the population calculated from self made observations of defecation. *J Epidemiol Commun H* 47:331-3.; 1993.
134. Rao, B. S. N. Pulses and Legumes as Functional Foods. *Nutrition Foundation of India (Publications) Current Bulletin* 2002: <http://www.nutritionfoundationofindia.org/PUBLICITN/Pub-cb1.html>; 2002.
135. Rao, P. S. Nature of carbohydrates in pulses. *J Agric Food Chem* 24:958-61; 1976.

136. Rao, P. V. *Statistical Research Methods in the Life Sciences*. Pacific Grove, California: Brooks/Cole Publishing Company; 1998.
137. Riccardi, G., Aggett, P., Brighenti, F., Delzenne, N., Frayn, K., Nieuwenhuizen, A., Pannemans, D., Theis, S., Tuijelaars, S., and Vessby, B. PASSCLAIM--body weight regulation, insulin sensitivity and diabetes risk. *Eur J Nutr* 43 Suppl 2:II7-II46; 2004.
138. Rimm, E. B., Ascherio, A., Giovannucci, E., Spiegelman, D., Stampfer, M. J., and Willett, W. C. Vegetable, fruit, and cereal fiber intake and risk of coronary heart disease among men. *JAMA* 275:447-51; 1996.
139. Rizkalla, S. W., Bellisle, F., and Slama, G. Health benefits of low glycaemic index foods, such as pulses, in diabetic patients and healthy individuals. *Br J Nutr* 88 Suppl 3:S255-62; 2002.
140. Robertson, T. L., Kato, H., Rhoads, G. G., Kagan, A., Marmot, M., Syme, S. L., Gordon, T., Worth, R. M., Belsky, J. L., Dock, D. S., Miyanishi, M., and Kawamoto, S. Epidemiologic studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California. Incidence of myocardial infarction and death from coronary heart disease. *Am J Cardiol* 39:239-43; 1977.
141. Sampath, H., and Ntambi, J. M. Polyunsaturated fatty acid regulation of gene expression. *Nutr Rev* 62:333-9; 2004.
142. Saris, W. H., Astrup, A., Prentice, A. M., Zunft, H. J., Formiguera, X., Verboeket-van de Venne, W. P., Raben, A., Poppitt, S. D., Seppelt, B., Johnston, S., Vasilaras, T. H., and Keogh, G. F. Randomized controlled trial of changes in dietary carbohydrate/fat ratio and simple vs complex carbohydrates on body weight and blood lipids: the CARMEN study. The Carbohydrate Ratio Management in European National diets. *Int J Obes Relat Metab Disord* 24:1310-8; 2000.
143. Schaefer, E. J. Lipoproteins, nutrition, and heart disease. *Am J Clin Nutr* 75:191-212; 2002.
144. Schaefer, E. J., Augustin, J. L., Schaefer, M. M., Rasmussen, H., Ordovas, J. M., Dallal, G. E., and Dwyer, J. T. Lack of efficacy of a food-

- frequency questionnaire in assessing dietary macronutrient intakes in subjects consuming diets of known composition. *Am J Clin Nutr* 71:746-51; 2000.
145. Schaefer, E. J., Lichtenstein, A. H., Lamon-Fava, S., Contois, J. H., Li, Z., Rasmussen, H., McNamara, J. R., and Ordovas, J. M. Efficacy of a National Cholesterol Education Program Step 2 Diet in Normolipidemic and Hypercholesterolemic Middle-Aged and Elderly Men and Women. *Arterioscler Thromb Vasc Biol* 15:1079-1085; 1995.
 146. Schaefer, E. J., Lichtenstein, A. H., Lamon-Fava, S., McNamara, J. R., Schaefer, M. M., Rasmussen, H., and Ordovas, J. M. Body weight and low-density lipoprotein cholesterol changes after consumption of a low-fat ad libitum diet. *JAMA* 274:1450-5; 1995.
 147. Schork, M. A., and Remington, R. D. *Statistics with Applications to the Biological and Health Sciences*. New Jersey: Prentice-Hall Inc; 2000.
 148. Schwenke, D. C. Insulin resistance, low-fat diets, and low-carbohydrate diets: time to test new menus. *Curr Opin Lipidol* 16:55-60; 2005.
 149. Setchell, K. D., and Cassidy, A. Dietary isoflavones: biological effects and relevance to human health. *J Nutr* 129:758S-767S; 1999.
 150. Setchell, K. D. R., Radd, S., Dreosti, I. E., Mann, J. I., Puddey, I., Worsley, A., Young, G. P., Wahlqvist, M., Okada, A., and Tanphaichitr, V. Soy and other legumes: 'bean' around a long time but are they the 'superfoods' of the millennium and what are the safety issues for their constituent phytoestrogens? *Asia Pac J Clin Nutr* 9:s13-s22; 2000.
 151. Sharma, R. D. Isoflavone content of Bengalgram (*Cicer arietinum*) at various stages of germination. *J Plant Food* 3:259-264; 1981.
 152. Shintani, T. T., and Hughes, C. K. Traditional diets of the Pacific and coronary heart disease. *J Cardiovasc Risk* 1:16-20; 1994.
 153. Shorey, R. L., Day, P. J., Willis, R. A., Lo, G. S., and Steinke, F. H. Effects of soybean polysaccharide on plasma lipids. *J Am Diet Assoc* 85:1461-5; 1985.

154. Siddiqui, M. T., and Siddiqui, M. Hypolipidemic principles of *Cicer arietinum*: biochanin-A and formononetin. *Lipids* 11:243-6; 1976.
155. Simpson, H. C., Simpson, R. W., Lousley, S., Carter, R. D., Geekie, M., Hockaday, T. D., and Mann, J. I. A high carbohydrate leguminous fibre diet improves all aspects of diabetic control. *Lancet* 1:1-5; 1981.
156. Singh, R. B., Rastogi, S. S., Niaz, M. A., Ghosh, S., Singh, R., and Gupta, S. Effect of fat-modified and fruit- and vegetable-enriched diets on blood lipids in the Indian Diet Heart Study. *Am J Cardiol* 70:869-74; 1992.
157. Singh, U., Subrahmanyam, N., and Kumar, J. Cooking quality and nutritional attributes of some newly developed cultivars of chickpea. *J Sci Food Agric* 55:37-46; 1991.
158. Slavin, J. L. Dietary fiber and body weight. *Nutrition* 21:411-8; 2005.
159. Soni, G. L., George, M., and Singh, R. Role of common Indian pulses as hypocholesterolaemic agents. *Ind J Nutr Dietet* 19:184-90; 1982.
160. Sri Kantha, S., and Erdman, J. W. J. Legume Carotenoids. *CRC Food Sci Nutr* 26:137-155; 1987.
161. Staniforth, D. H., Baird, I. M., Fowler, J., and Lister, R. E. The effects of dietary fibre on upper and lower gastro-intestinal transit times and faecal bulking. *J Int Med Res* 19:228-33; 1991.
162. Stephen, A. M., Dahl, W. J., Johns, D. M., and Englyst, H. N. Effect of Oat Hull Fiber on Human Colonic Function and Serum Lipids. *Cereal Chem* 74:379-383; 1997.
163. Stephen, A. M., Dahl, W. J., Sieber, G. M., van Blaricom, J. A., and Morgan, D. R. Effect of green lentils on colonic function, nitrogen balance, and serum lipids in healthy human subjects. *Am J Clin Nutr* 62:1261-7; 1995.
164. Storlien, L. H., Higgins, J. A., Thomas, T. C., Brown, M. A., Wang, H. Q., Huang, X. F., and Else, P. L. Diet composition and insulin action in animal models. *Br J Nutr* 83 Suppl 1:S85-90; 2000.

165. SUGiRS Sydney University's Glycaemic Index Research Unit: Glycaemic Index Research Report for Simplot Australia. Sydney NSW: Human Nutrition Unit, Department of Biochemistry, Sydney University; 2003.
166. Sukhminder, K., Uberoi, S., Vadhera, and Soni, G. L. Role of dietary fibre from pulses and cereals as hypocholesterolaemic and hypolipidaemic agent. *J Food Sci Technol* 29:281-283; 1992.
167. Tharanathan, R. N., and Mahadevamma, S. Grain legumes - a boon to human nutrition. *Trends in Food Science & Technology* 14:507-518; 2003.
168. Tong, B. C., and Barbul, A. Cellular and physiological effects of arginine. *Mini Rev Med Chem* 4:823-32; 2004.
169. Trowell, H. Ischemic heart disease and dietary fiber. *Am J Clin Nutr* 25:926-32; 1972.
170. Truswell, A. S. Dietary fiber and health. *World Rev Nutr Diet* 72:148-64; 1993.
171. Urizar, N. L., and Moore, D. D. Gugulipid: A Natural Cholesterol-Lowering Agent. *Annu Rev Nutr*; 2003.
172. USDA Nutrient Database for Standard Reference, Release 15. In: USDA (ed.), *USDA Nutrient database for Standard reference 2002*: http://www.nal.usda.gov/fnic/cgi-bin/list_nut.pl; 2002.
173. Uusitupa, M., Lindi, V., Louheranta, A., Salopuro, T., Lindstrom, J., and Tuomilehto, J. Long-term improvement in insulin sensitivity by changing lifestyles of people with impaired glucose tolerance: 4-year results from the Finnish Diabetes Prevention Study. *Diabetes* 52:2532-8; 2003.
174. Van Gaal, L. F., Mertens, I. L., and Ballaux, D. What is the relationship between risk factor reduction and degree of weight loss? *Eur Heart J Suppl* 7:L21-L26; 2005.
175. Van Horn, L. Fiber, lipids, and coronary heart disease. A statement for healthcare professionals from the Nutrition Committee, American Heart Association. *Circulation* 95:2701-4; 1997.

176. Verschuren, W. M., Jacobs, D. R., Bloemberg, B. P., Kromhout, D., Menotti, A., Aravanis, C., Blackburn, H., Buzina, R., Dontas, A. S., and Fidanza, F. Serum total cholesterol and long-term coronary heart disease mortality in different cultures. Twenty-five-year follow-up of the seven countries study. *JAMA* 274:131-6; 1995.
177. Vessby, B. Dietary fat and insulin action in humans. *Br J Nutr* 83 Suppl 1:S91-6; 2000.
178. Visscher, T. L., and Seidell, J. C. The public health impact of obesity. *Annu Rev Public Health* 22:355-75; 2001.
179. Wald, N. J., and Law, M. R. Serum cholesterol and ischaemic heart disease. *Atherosclerosis* 118 Suppl:S1-5; 1995.
180. Wannamethee, S. G., Shaper, A. G., and Walker, M. Overweight and obesity and weight change in middle aged men: impact on cardiovascular disease and diabetes. *J Epidemiol Commun H* 59:134-9; 2005.
181. Westerterp-Plantenga, M. S., and Lejeune, M. P. Protein intake and body-weight regulation. *Appetite* 45:187-90; 2005.
182. WHO/FAO Diet, nutrition and prevention of chronic diseases: Report of a joint WHO/FAO expert consultation, Geneva, 28Jan - 1 Feb 2002. Geneva; 2003.
183. Wijendran, V., and Hayes, K. C. Dietary n-6 and n-3 fatty acid balance and cardiovascular health. *Annu Rev Nutr* 24:597-615; 2004.
184. Williams, P. C., and Singh, U. Nutritional quality and the evaluation of quality in breeding programs. In: M. C. Saxena and K. B. Singh (eds.), *The Chickpea*, pp. 329-356. Wallingford, Oxon, U.K.: C.A.B. International; 1987.
185. Wing, R. R., Koeske, R., Epstein, L. H., Nowalk, M. P., Gooding, W., and Becker, D. Long-term effects of modest weight loss in type II diabetic patients. *Arch Intern Med* 147:1749-53; 1987.
186. Wu, G., and Meininger, C. J. Arginine nutrition and cardiovascular function. *J Nutr* 130:2626-9; 2000.

187. Wynne, K., Stanley, S., McGowan, B., and Bloom, S. Appetite control. *J Endocrinol* 184:291-318; 2005.
188. Yao, M., and Roberts, S. B. Dietary energy density and weight regulation. *Nutr Rev* 59:247-58; 2001.
189. Yusuf, S., Hawken, S., Ounpuu, S., Dans, T., Avezum, A., Lanas, F., McQueen, M., Budaj, A., Pais, P., Varigos, J., and Lisheng, L. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet* 364:937-52; 2004.
190. Zhan, S., and Ho, S. C. Meta-analysis of the effects of soy protein containing isoflavones on the lipid profile. *Am J Clin Nutr* 81:397-408; 2005.
191. Zulet, M. A., Macarulla, M. T., Portillo, M. P., Noel-Suberville, C., Higuieret, P., and Martinez, J. A. Lipid and glucose utilization in hypercholesterolemic rats fed a diet containing heated chickpea (*Cicer aretinum* L.): a potential functional food. *Int J Vitam Nutr Res* 69:403-11; 1999.
192. Zulet, M. A., and Martinez, J. A. Corrective role of chickpea intake on a dietary-induced model of hypercholesterolemia. *Plant Foods Hum Nutr* 48:269-77; 1995.