LEGUME INFLAMMATION FEEDING EXPERIMENT (LIFE): EFFECTS ON LIPIDS AND LIPOPROTEINS, LEPTIN AND GHRELIN

A Dissertation in Nutrition

by

Zhiying Zhang

© 2010 Zhiying Zhang

Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

August 2010
The dissertation of Zhiying Zhang was reviewed and approved by the following:

Terryl J. Hartman  
Association Professor of Nutritional Sciences  
Dissertation Advisor  
Chair of Committee

Mosuk Chow  
Senior Research Associate and Associate Professor

A. Catharine Ross  
Professor and Occupant of Dorothy Foehr Huck Chair

Penny M. Kris-Etherton  
Distinguished Professor of Nutritional Sciences

Jan S. Ulbrecht  
Professor of Biobehavioral Health and Medicine

Gordon Jensen  
Professor and Department Head of Nutritional Sciences

*Signatures are on file in the Graduate School
ABSTRACT

Colorectal cancer is the second leading cause of cancer death among men and women in the U.S. Epidemiological investigations show that nutritional-related conditions such as diets high in fat, low in fruits and vegetables, and physical inactivity, may play a vital role in cancer development. Recently, a large multi-center, randomized trial, the Polyp Prevention Trial, with 1905 subjects who had recently removed colorectal adenomas showed that high legume consumption, independent of fruit and vegetable intake, was associated with significantly lower risk for adenoma recurrence. We employed a randomized crossover controlled-feeding study (The Legume Inflammation Feeding Experiment) to evaluate the effects a legume enriched, high fiber, low glycemic index diet (LG) on biomarkers of insulin resistance and inflammation in men with high risk for colorectal cancer. Very important objectives were to evaluate the effects on lipids and lipoproteins, cholecystokinin, leptin, and ghrelin. Serum total cholesterol (TC), LDL cholesterol (LDL-C), triglycerides (TG), and plasma leptin levels significantly decreased after the LG diet (P<0.0001 for TC, LDL-C, and leptin, and P=0.0028 for TG). In addition, we also found that insulin-resistant individuals did not achieve the full benefit of the LG diet on the HDL cholesterol (HDL-C) related ratios. In conclusion, this study demonstrates that high legume consumption elicit beneficial effects on cardiovascular disease (CVD) risk factors and weight management.
# TABLE OF CONTENTS

**LIST OF TABLES** .................................................................................................................. vi

**LIST OF FIGURES** .................................................................................................................. vii

**ACKNOWLEDGEMENTS** ......................................................................................................... viii

**Chapter 1 Introduction & Literature Review** ......................................................................... 1

Epidemiology of cardiovascular disease, metabolic syndrome/ insulin resistance syndrome, and inflammation .......................................................... 3
  Insulin resistance syndrome and CVD ................................................................. 4
  Inflammation, CVD, and colorectal cancer ....................................................... 4
  Glycemic index and glycemic load ................................................................... 5
  Legume definition and the diversity of foods ................................................... 6
The effects of legume components on CVD ......................................................... 7
  Dietary fiber ........................................................................................................ 7
  Plant stanols and sterols .................................................................................... 8
  Folate .................................................................................................................. 9
  Antioxidants ....................................................................................................... 10
  Minerals ............................................................................................................... 11
    Calcium ............................................................................................................ 11
    Magnesium ..................................................................................................... 12
    Potassium ......................................................................................................... 13
The effects of legume components on satiety and weight management .......... 14
  Dietary fiber ........................................................................................................ 14
  Trypsin inhibitor ............................................................................................... 15
  Energy density .................................................................................................... 15
Mechanisms of the cholesterol-lowering effects and the satiety effects of legumes ................................................................................... 16
  Short-chain fatty acid (SFCA) and cholesterol synthesis .............................. 16
  Changes in plasma CCK, leptin, and ghrelin .................................................. 17
Review of clinical studies with legumes and CVD/ metabolic syndrome .... 19
References ................................................................................................................. 21

**Chapter 2 A High Legume Low Glycemic Index Diet Improves Serum Lipid Profiles in Men** ......................................................................................................................... 29

Abstract ......................................................................................................................... 29
Introduction .................................................................................................................... 31
Subjects and methods ............................................................................................... 32
  Subjects and recruitment .................................................................................... 32
  Criteria and restrictions ....................................................................................... 32
  Diet and study design .......................................................................................... 33
  Sample collection and measurements ............................................................... 34
Chapter 3 The Effects of a High Legume Low Glycemic Index Diet on Fasting Plasma Leptin and Ghrelin in Older Men .................................52

Abstract .................................................................52
Introduction ...............................................................54
Subjects and methods ..................................................56
  Subjects and recruitment ..............................................56
  Criteria and restrictions ...............................................56
  Diet intervention and study design .................................57
  Sample collection and measurement ...............................58
  Statistical analysis ....................................................58
Results .........................................................................59
  Baseline characteristics ..............................................59
  Effects of experimental diets on fasting leptin and ghrelin ....60
  Effects of experimental diets on fasting leptin and ghrelin stratified by insulin sensitivity status ..........................60
  Associations among the changes in study variables ..........61
Discussion .................................................................61
References ......................................................................61

Chapter 4 Summary, Strengths, Limitations and Future Directions ..........81

Summary .................................................................81
Strengths .................................................................83
Limitations ...............................................................84
  Potentially subjective classification of insulin sensitivity/resistance ......84
  Inference for polyp recurrence .......................................85
  Short compliance breaks .............................................85
Future directions .........................................................86
  Dose-response of high legume consumption ....................86
  Post-meal effect and time course ...................................86
  Other gut hormone candidates on satiety .........................87
  Target group and possible study designs .........................88
References .....................................................................89

Appendix A Informed Consent Form for Clinical Research Study ............91
Appendix B Informed Consent Form for Clinical Research Study – Addendum to Primary Consent Form ........................................99
Appendix C Telephone Interview Form .......................................................102
Appendix D Bean Recipes .................................................................107
Appendix E Menus of the Healthy American Diet and the Legume Diet ...120
Appendix F Plots of Changes in Study Outcomes on Two Diets ............122
LIST OF TABLES

Table 1-1 Nutrients of Selected Legumes ........................................28
Table 2-1 Nutrient and Food Profile of the Experimental Diets (Compared to Pre-study Diet) .................................................................46
Table 2-2 Baseline Characteristics of Study Subjects by Insulin Resistance Status ......47
Table 2-3 Effects of Diets on Lipids and Lipoproteins ................................48
Table 2-4 Effects of Diets on Lipids and Lipoproteins among Subjects Stratified by Diet .................................................................49
Table 3-1 Nutrient and Food Profile of the Experimental Diets (Compared to Pre-study Diet) .................................................................67
Table 3-2 Baseline Characteristics of Study Subjects ................................68
Table 3-3 Effects of Diets on Leptin and Ghrelin ....................................69
Table 3-4 Effects of Diets on Leptin and Ghrelin among Subjects Stratified by Diet .................................................................70
LIST OF FIGURES

Figure 2-1 Relationship between HOMA-IR index at study entry and differences in TC:HDL cholesterol ratio changes (Delta TC:HDL-C ratio) on the two diets ..........................................................51

Figure 3-1 Positive association between changes in fasting glucose and changes in fasting leptin during the LG diet ..........................................................71

Figure 3-2 Positive association between changes in fasting insulin and changes in fasting leptin during the LG diet ..........................................................72

Figure 3-3 Positive association between changes in fasting C-peptide and changes in fasting leptin during the LG diet ..........................................................73

Figure 3-4 Negative association between changes in fasting C-peptide and changes in fasting ghrelin during the LG diet ..........................................................74

Figure 3-5 Positive association between changes in fasting glucose and changes in fasting leptin during the HA diet ..........................................................75

Figure 3-6 Positive association between changes in fasting insulin and changes in fasting leptin during the HA diet ..........................................................76

Figure 3-7 Positive association between changes in fasting C-peptide and changes in fasting leptin during the HA diet ..........................................................77
ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincere and special appreciation to my advisor and committee chair, Dr. Terryl Hartman, for your patience and contribution. My research is getting better and better with your endless encouragement and guide. I am very happy to have you to work with and my life at Penn State is always brightened because of you. I also would like to gratefully thank other committee members: Drs. Chow, Ross, Kris-Etherton, and Ulbrecht. Without you, I would not have been able to do this work. I obtained so much academic knowledge, friendship, warmth, and encouragement from you. Dr. Chow, my statistics minor advisor, is always there when I need help with statistics. Dr. Ross, my laboratory supervisor, guides me with all laboratory measurements and makes me learn so much advanced knowledge on gut hormone regulation and metabolism. Dr. Kris-Etherton and Dr. Ulbrecht, two of the LIFE Study co-principle investigators, have been helping me with data interpretation and manuscript writing. With the help from you and Dr. Ross, my writing is more logical and scientific.

I would like to express my sincere gratitude to Dr. Michael Rovine, who helps me with model selection and data analyses. I am deeply grateful to Mrs. Dee Bagshaw, Diane Mitchell, and Linda Phelps. Dee gives me guidance about how to be an excellent study coordinator. Without your help, I would not have known how to complete recruitment, sample processing, participant management, and data interpretation. Diane and Linda help me with 24-hour dietary recalls and all diet-related data analyses. Data input and analyses are very time-consuming but you are always there to support me. I would like to
thank Dr. Hartman’s research group and staff of the General Clinical Research Center and the faculty, staff especially Stacie Hugney and Judy Jones, and graduate students of the Department of Nutritional Sciences. I had a great time here in the Happy Valley with your warm friendship and endless help. My life here becomes much more colorful because of you all.

Finally, I would like to express my deep appreciation to my family for the unconditional support. In particular, my parents, who give me warm and powerful love and mean so much to me. Without you, mom and dad, my wishes would not have come true. I also would like to thank my husband, Yuefeng, and my son, Yuran, for your love and support.
Chapter 1

Introduction and Literature Review

Colorectal cancer (CRC) is the second leading cause of cancer death in the United States (1). Although the specific causes of colorectal cancer are still unclear, epidemiological and experimental studies suggest that nutritional-related factors may play a vital role in cancer development. Diets high in fat and low in fruits, vegetables, and fiber, and physical inactivity may all be important risk factors. Interestingly, people with many of these unhealthy behaviors are more likely to be overweight and insulin-resistant, and to be at increased risk for cardiovascular disease (CVD).

Weight loss may be an important contributor to chronic disease risk. One of the approaches to weight loss is to reduce energy intake. Dietary fiber may favorably affect diet energy density (ED), or the amount of energy in a given food weight (e.g. kcal/g), and may efficiently reduce human energy intake through satiety. Many epidemiological studies have shown inverse associations between fiber consumption and the risk for chronic disease such as CVD (2-6), type 2 diabetes (7-10), and CRC (11-14).

Legumes are good sources of dietary fiber, particularly soluble fiber which becomes gel-like during digestion. The viscous substance may delay cellular glucose uptake and release into the blood stream; thus lowering postprandial levels of glucose and insulin. Additionally, the colonocytes use fermented soluble fiber as their energy source and produce a large amount of short-chain fatty acids (SCFAs), especially butyrate. Butyrate plays an important role in suppressing cholesterol synthesis and may protect the colonocytes from carcinogenesis. Insoluble fiber reduces the transit time of waste in the
large intestine and softens feces. Legumes also are good sources of nutritional factors that have been shown to exert cardioprotective effects including folate, flavonoids, and several minerals such as calcium, magnesium, and potassium (Table 1-1).

It is well established that elevated circulating total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), and triglyceride (TG) levels contribute to CVD development whereas elevated high density lipoprotein cholesterol (HDL-C) levels reduce CVD risk. More recently, biomarkers such as C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) have been associated with inflammation (15,16). However, no specific biomarkers have been identified for CRC.

Recently, a large multi-center, randomized trial (Polyp Prevention Trial, PPT) (17) of 1905 subjects with colorectal adenomas showed that high legume consumption, independent of fruit and vegetable intake, was significantly associated with lower risk for adenoma recurrence. The Legume Inflammation Feeding Experiment (LIFE Study) was the first randomized, controlled cross-over human feeding study to evaluate the effects of a high legume low glycemic index (GI) diet on biomarkers of insulin resistance and inflammation in males with higher risk for colorectal cancer. Primary study objectives were to evaluate the effects of the high legume diet on serum CRP and C-peptide, an indicator of insulin resistance. Secondary objectives were to evaluate the diet effects on additional inflammatory biomarkers such as IL-6 and TNF-α, fecal SCFAs, and serum lipids and lipoproteins. Tertiary objectives were to evaluate the diet effects on satiety through changes in gut hormones, cholecystokinin (CCK), leptin, and ghrelin.

Collectively, the current study was designed to test whether the legume-enriched diet
ameliorates insulin resistance and inflammation and whether the diet lowers the risk for CVD and CRC.

**Epidemiology of cardiovascular disease, metabolic syndrome/insulin resistance syndrome, and inflammation**

Cardiovascular disease, including coronary heart disease (CHD), stroke, and hypertension, is the leading cause of death in the United States (18). Approximately 81 million American adults live with some form of CVD (18). According to the American Heart Associations (AHA) updated “Heart Disease and Stroke Statistics” (19), deaths from CVD (861,826) were 35.2% of total deaths in 2005.

Metabolic syndrome (MS) is a cluster of health disorders, including abdominal obesity, atherogenic dyslipidemia, elevated blood pressure, insulin resistance and/or glucose intolerance, and prothrombotic and proinflammatory states (20). The Third Report of National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III, NHLBI) identifies people with three or more of the following criteria as MS patients: 1) abdominal obesity – waist circumference > 102 cm in men and > 88 cm in women, 2) hypertriglyceridemia – serum TG ≥ 150 mg/dL (1.69 mmol/L), 3) low HDL-C – < 40 mg/dL (1.04 mmol/L) in men and < 50 mg/dL (1.29 mmol/L) in women, 4) hypertension – blood pressure ≥ 130/85 mmHg, and 5) high fasting glucose – ≥ 110 mg/dL (6.1 mmol/L) (20). Insulin resistance (IR), a metabolic disorder where the body cannot recognize and use insulin efficiently, is interchangeable with MS. According to the National Health and Nutrition Examination
Survey (NHANES) 1999-2000, the age-adjusted prevalence of MS was 27% among U.S. adults (21). People with MS are at high risk for chronic diseases, such as CVD (22), type 2 diabetes (23), obesity (24), and several cancers including colorectal cancer (25).

**Insulin resistance syndrome and CVD**

Insulin resistance blunts glucose uptake and increases the mobilization of free fatty acids from adipose tissues. These alterations increase hepatic synthesis of very-low-density lipoproteins (VLDL); lipoprotein lipase expression is diminished and TG-rich VLDL is built up. The buildup promotes the production of TG-rich small dense HDL and small dense LDL particles. All together, the deposition of cholesterol within the artery wall increases and contributes to atherogenesis (26).

Many epidemiological studies suggest that IR is an important predictor for CVD. A review of prospective studies from 1998 through 2004 (27) showed that people with this syndrome are about twice likely to die of CVD (adjusted relative risk, RR=1.65, 95% CI =1.38-1.99). In the Health, Aging, and Body Composition (Healthy ABC) study (28) of 3,035 men and women aged 70-79 years, participants with this syndrome had a significantly higher risk for coronary events (adjusted hazard ratio, HR=1.56, 95% CI=1.28-1.91). In the Kuopio Ischadmic Heart Disease Risk Factor Study (29) of 1209 middle-aged Finnish men, results after nearly 12 years of follow-up showed that risk of death from CVD among men with this syndrome was nearly doubled (adjusted RR=1.92, 95% CI=0.91-4.07).

**Inflammation, CVD, and colorectal cancer**
It has been well established that inflammation plays a vital role in the pathogenesis of atherosclerosis (30,31). Inflammation increases production of many cytokines such as TNF-α, IL-1 and IL-6, and CRP from hepatocytes and macrophages. In particular, TNF-α and IL-6 activate the expression of endothelial adhesion molecules, which facilitate the attachment of monocytes to endothelial cells and the migration into artery walls. The transformation of monocytes into macrophages, uptake of oxidized LDL, and formation of foam cells initiate fatty streaks. With the accumulation of macrophages, oxidized lipids, and debris in the lesion, the smooth muscle cells undergo apoptosis contributing to the growth of atherosclerotic plaque (30,32). Several epidemiological studies have shown positive associations between elevated levels of CRP (33,34), IL-6 (33,35), and TNF-α (36) and cardiovascular events.

Inflammation also contributes to increased risk for carcinoma. For example, TNF-α stimulates the activation of κB kinase β (IKKβ), an upstream activator of NF-κB. NF-κB stimulates cell proliferation and depresses differentiation and apoptosis, which may ultimately result in colorectal tumorgenesis (37). Positive associations between the aforementioned biomarkers and CRC risk have been shown in both clinical (38) and epidemiological (39) studies.

**Glycemic index and glycemic load**

Carbohydrate-containing foods may be categorized by glycemic index (GI). GI is determined by assessing the increase in blood glucose level caused by ingestion of 50 grams of a carbohydrate-containing food compared to that of 50 grams of glucose (40). Glycemic load (GL) of a food is determined by multiplying the GI by the amount of
carbohydrate in grams provided by the food and dividing by 100 (41). Excessive intake of foods with a high GI raises postprandial blood glucose dramatically and over the long-term may lead to hyperinsulinemia and insulin resistance. Glucose ingestion may also stimulate the secretion of circulating inflammatory cytokines such as TNF-α, IL-6, and IL-18 (42) as well as proinflammatory transcription factors such as NF-κB (43).

Epidemiological studies show positive associations between GI and/or glycemic load (GL) and chronic diseases including heart disease, diabetes and colorectal cancer. In the Nurses’ Health Study cohort (44) of 75,521 women aged 38-63 years old with no previous diagnosis of diabetes or heart disease events, both GI (adjusted RR=1.31, 95% CI=1.02-1.68, P for trend=0.008) and GL (adjusted RR=1.98, 95% CI=1.41-2.77, P for trend <0.0001) were positively associated with CHD risk during follow-up. In another prospective cohort (45) of 15,714 Dutch women aged 49-70 years without diabetes or CVD at baseline, both GI and GL were positively associated with CVD (adjusted HR for GI =1.33, 95% CI=1.07-1.67, P for trend=0.021; adjusted HR for GL=1.47, 95% CI=1.04-2.09, P for trend=0.03). In relation to inflammatory biomarkers, a cross-sectional analysis (46) of 18,137 subjects from the Women’s Health Study showed that GI was significantly positively associated with fasting CRP.

**Legume definition and the diversity of foods**

Legumes generally have low GI values (47) and have been used as substitutes for higher GI foods such as rice and potatoes. Legumes are the plants of the family *Fabaceae* or *Leguminosae* or the fruits of these plants that are used for food. Well-known legumes include peas, beans, lentils, lupins, soybeans, and peanuts. Legumes are nutrient-dense
foods, which are rich in essential fatty acids, plant protein, dietary fiber, phytochemicals, and minerals. Although legumes are an important type of food around the world, the consumption in Western diets is low (48). The 2005 Dietary Guideline for Americans recommends an average intake of 14 g/d legumes for people who consume 2,000 kcal as a strategy to lower the risk for chronic diseases such as obesity, type 2 diabetes, CVD, and colorectal cancer.

This dissertation focuses on analyses designed to evaluate the effects of the legume-enriched low GI diet on CVD risk and satiety; the remainder of this review will focus on the effects of relevant legume components.

The effects of legume components on CVD

Dietary fiber

Dietary fiber can be sub-divided into soluble (pectins, gums, β-glucans, and mucilages) and insoluble fiber (celluloses, lignans, and hemicelluloses) (49). Insoluble fiber is high in vegetables, especially legumes, and reduces the transit time of waste in the large intestine and softens feces. Soluble fiber, high in fruits, vegetables, and oats, becomes gel-like during digestion; the viscous substances may delay glucose release into the blood stream, theoretically lowering postprandial levels of glucose (50) and insulin (51). Additionally, the colonocytes in the large intestine digest fermented soluble fiber as their energy source, producing a large amount of SCFAs, especially butyrate, which plays a vital role in suppressing cholesterol synthesis (52,53).

Numerous epidemiological studies have shown an inverse association between dietary fiber intake and CVD risk. In the Alpha-Tocopherol, Beta-Carotene Cancer
Prevention Study cohort (the ATBC Study) (5) of 21,930 older smoking men dietary fiber intake was strongly inversely associated with CHD after six years of follow-up. Men in the highest quintile of total dietary fiber intake had approximately 30% lower risk for coronary death (adjusted RR=0.69, 95% CI=0.54-0.88, P for trend <0.001). Similar associations were also observed in the Nurses’ Health Study cohort (54) and a meta-analysis of 10 studies conducted in the U.S. and Europe (6). Larsson et al. (55) analyzed the same population from the ATBC Study and observed a similar beneficial effect of vegetable fiber intake on cerebral infarction. In the Cardiovascular Health Study (56) of 3,588 men and women aged ≥65 years from 1989 to 2000, higher intake of cereal fiber was significantly associated with lower risks of total and ischemic stroke (adjusted HR=0.79, 95% CI=0.62-0.99, P for trend=0.02). Rimm et al. (3) reported results from a large cohort of middle-aged male health professionals. A 10g/d increment of total dietary fiber from vegetable, fruit, and cereal contributed significantly lower risk for myocardial infarction (MI; adjusted RR=0.81, 95% CI=0.70-0.93) after six years of follow-up. A similar association was observed in the Zutphen Study (57); an increment of 10 g/d of dietary fiber intake significantly reduced CHD mortality (17%, 95% CI=2-30%) and all-cause mortality (9%, 95% CI=0-18%).

**Plant stanols and sterols**

Phytosterols, stanols and sterols, are primarily present in nuts, vegetable oils, seeds, cereals, and legumes (58). The absorption rates of plant stanols (0.02-0.3%) and sterols (0.4-3.5%) in the small intestine are very low compared to that of cholesterol (35-70%); possibly due to the low affinity of acyl-Co A cholesterol acyltransferase (ACAT)
Plant stanols and sterols have similar chemical structures to cholesterol and affect cholesterol absorption by enterocytes. They are more hydrophobic than cholesterol, which favors the replacement of cholesterol and reduces cholesterol concentrations in the micelles (60). Plant stanols or sterols may reduce the esterification rate of cholesterol in the enterocytes (61). Decreased cholesterol absorption stimulates cholesterol synthesis (62) as well as the expression of LDL receptor mRNA (63) increasing LDL clearance and lowering LDL production resulting in lower circulating total cholesterol levels. Collectively, because of their cholesterol-lowering effects, plant stanols and sterols have been incorporated into food products in food industry.

Folate

Legumes are good sources of folate providing 130-170 μg per 100g on average. Folate coenzymes play essential roles in many one-carbon metabolism reactions such as amino acid metabolism including histidine, serine, glycine, and methionine, as well as purine and pyrimidine synthesis. Folate is required for the synthesis of methionine from homocysteine. Folate deficiency causes homocysteine buildup, which may lead to blood clotting and artery wall thickening (64), thus increasing CVD risk (65).

A meta-analysis of 30 observational studies of 16,786 subjects (66) showed that a 25% reduction in plasma homocysteine level was significantly associated with decreased risks for both ischemic heart disease (IHD; adjusted OR=0.89, 95% CI=0.83-0.96) and stroke (adjusted OR=0.81, 95% CI=0.69-0.95). In the Women’s Antioxidant and Folic Acid Cardiovascular Study (WAFACS) (67) of 5,442 middle-aged female health professional, folate supplementation (2.5 mg of folic acid combined with 50 mg of
vitamin B₆ and 1 mg of vitamin B₁₂) resulted in a significant reduction in plasma homocysteine levels after seven years, yet total cardiovascular events were unchanged.

**Antioxidants**

Oxidative stress (OS) is involved in many human chronic diseases such as Parkinson’s disease and Alzheimer’s disease (68,69), CVD (70,71), and cancer (72,73). OS primarily results from the excessive production of reactive oxygen species, including oxygen ions and peroxides that cause a variety of alterations such as lipid oxidation and DNA methylation. Selenium-dependent glutathione peroxidase and copper-dependent superoxide dismutase are produced by the human body to guard against OS, and many other antioxidants like vitamin C, vitamin E, carotenoids, and polyphenols that are rich in fruits and vegetables are considered as secondary antioxidants against OS and in the prevention of chronic diseases (74,75).

Legumes are rich in a variety of bioactive compounds such as flavonoids from the polyphenol family. Cho et al. (76) compared 21 fruits, 67 vegetables, and 7 legumes (black beans, cowpeas, kidney beans, mung beans, peas, small red beans, and soy beans) and reported that the levels of total phenols in the beans were higher (1,370±589.9 mg gallic acid equivalent, GAE/kg FW) than the yellow, orange, and red vegetables and light colored vegetables.

The association between polyphenol intake and CVD is controversial. In the large population-based Kuopio Ischaemic Heart Disease Risk Factor Study of 1,950 Finnish men aged 42-60 years (77), 26 flavonoids from five subclasses (flavonols, flavones, flavanones, flavan-3-ols, and anthoryanidins) were evaluated for associations with
ischaemic stroke and CVD mortality. Men in the highest quintile of flavonol and flavanone consumption had adjusted RRs of 0.55 (95% CI=0.31-0.99) and 54 (95% CI=0.32-0.92), respectively for ischaemic stroke, compared to those in the lowest quintiles. In the Iowa Women’s Health Study (78) of 34,489 postmenopausal women, subjects who were in the highest quintile of dietary flavanone intake had 21% lower risk for CHD (adjusted RR=0.78, 95% CI=0.65-0.94) compared to the lowest quintile. However, in a randomized double-blind placebo-controlled trial (79), 6-month isoflavone supplementation (70 mg) did not significantly change lipid profiles, fasting glucose, fasting insulin, or blood pressure and the study concluded that isoflavone consumption did not favorably reduce CVD risk compared with placebo. Further research is needed to clarify whether the inconsistencies in results observed between studies are due to the types of polyphenols investigated, time course, or the variability in total amounts of polyphenols tested.

**Minerals**

Legumes are also good sources of minerals such as calcium, magnesium, manganese, and potassium. Metabolic studies indicate that the beneficial effects may result from the improvement of lipid profile and blood pressure.

**Calcium**

Legumes provide 30 - 50 mg calcium per 100g on average. Epidemiological studies show an inverse association between calcium (Ca) intake and risk for CVD. In a prospective study of 41,526 Japanese men and women aged 40-59 years without a history
of CVD or cancer (80), people in the highest quintile of total Ca consumption had 30% lower CVD risk compared to those in the lowest quintile (adjusted HR=0.70, 95% CI=0.56-0.88, P for trend=0.02). Dairy Ca consumption was inversely associated with the risk of ischemic stroke (adjusted HR=0.69, 95% CI=0.52-0.93, P for trend=0.05). Experimental studies also indicate that Ca intake may improve lipid profiles. In a cross-over study (81) of 31 healthy young adults, the consumption of 1g Ca/d (supplied as pentacalcium hydroxyl-triphosphate into bread) over four weeks significantly lowered TC (-9mg/dL, P=0.008) compared to the control (non-supplemented bread).

The association between Ca intake and blood pressure is controversial (82,83). In a meta-analysis of 42 randomized clinical trials (84), dietary (9 studies) and nondietary (33 studies) calcium supplementation significantly reduced both systolic blood pressure (SBP; -1.44 mm Hg, 95% CI= -2.20 to -0.68) and diastolic blood pressure (DBP; -0.84 mm Hg, 95% CI= -1.44 to -0.24). More recently, the Women’s Health Study (85) reported that women who were in the highest quintile of dietary calcium intake had 13% lower risk for hypertension compared to those in the lowest quintile (95% CI= 0.81-0.93, P for trend <0.0001), whereas supplemental calcium had no significant effects on hypertension prevention (adjusted RR=1.07; 95% CI=0.97-1.18). However, in the Women’s Health Initiative Calcium/Vitamin D Trial (86) of 36,282 postmenopausal participants followed seven years, daily intake of 1,000 mg of elemental calcium with 400 IU of vitamin D₃ did not significantly reduce SBP or DSP.

**Magnesium**
Legumes provide 40-50 mg of magnesium (Mg) per 100g on average. Epidemiological studies indicate that magnesium (Mg) consumption may affect CVD risk. In a prospective study (87) of 39,876 female health professionals aged 39-89 years followed for 10 years, there was no association between Mg consumption and CHD prevention but hypertensive incidents (88). Data from the Women’s Health Study cohort supported these results. Women in the highest quintile of Mg intake had a decreased risk for hypertension (adjusted RR=0.87, 95%CI=0.81-0.93, P for trend<0.0001) compared to those in the lowest quintile.

**Potassium**

Legumes are also good sources of potassium, providing 350 - 400 mg per 100 g on average. It is well-established that dietary potassium and sodium contribute to the control of blood pressure; however, the underlying mechanisms are still unclear. Oberleithner et al. (89) reviewed several molecular studies and hypothesized that sodium and potassium may be involved in the “solat-gelation” alteration of endothelial cells. Briefly, endothelial cells are targets for aldosterone, a hormone that stimulates the apically located epithelial sodium channel. With the presence of aldosterone, high plasma sodium gelates endothelial cell membrane, increases cell stiffness, and thus causes hypertension. In contrast, high plasma potassium softens and fluidizes the membrane, which lowers blood pressure.

Several meta-analyses have evaluated the associations between potassium and sodium consumption with blood pressure. Cappuccio et al. (90) reviewed 19 clinical trials of 586 men and women across North America, Europe, Asia, and Africa. They observed
that potassium supplement consumption (~50 up to 140 mmol/d) significantly lowered both SBP (-5.9 mmHg, 95% CI= -6.6 to -5.2 mmHg) and DBP (-3.4 mmHg, 95% CI= -4.0 to -2.8 mmHg). Whelton et al. (91) reviewed 33 randomized clinical controlled trials of 2,609 subjects and reported similar results. Potassium supplementation was associated with the reductions of both SBP (-3.11 mmHg, 95% CI= -4.31 to -1.91 mmHg) and DBP (-1.97 mmHg, 95% CI= -3.42 to -0.52 mmHg).

**The effects of legume components on satiety and weight management**

Legumes may have effects on satiety and weight management through a number of mechanisms involving dietary fiber, trypsin inhibitor (a protease inhibitor), and energy density. Both animal and human clinical studies indicate that all of them play important roles.

**Dietary fiber**

Dietary fiber may contribute to body weight regulation through a number of plausible physiological mechanisms. Soluble fiber becomes gel-like during digestion, macronutrient absorption may be decreased and a long-term imbalance between energy intake and energy expenditure may lead to weight loss. Another possible mechanism is that high fiber intake may delay gastric emptying thus extending the feeling of fullness through the regulation of certain gut hormones such as cholecystokinin (CCK), leptin, and ghrelin.

Many epidemiological studies have shown beneficial effects of high fiber intake on weight loss among overweight and obese people (92,93). In a prospective cohort study
of 74,091 female nurses aged 38-63 years women who consumed more whole grains had significantly lower body weight compared to those who consumed less whole grain after six years of follow-up. Women subjects in the highest quintile of dietary fiber intake had a 49% lower risk for weight gain compared to those with the lowest dietary fiber intakes (adjusted OR=0.51; 95% CI=0.39-0.67; P for trend <0.0001). Howarth et al. (49) reviewed 11 manuscripts on the effects of dietary fiber on hunger, satiety, energy intake, and body composition among healthy populations and concluded that an additional 14 g/day of fiber intake resulted in 1.9 kg of weight loss over 3.8 months when energy intake was ad libitum.

**Trypsin inhibitor**

During a meal digestion proteins and fats that enter the small intestine stimulate CCK secretion into the blood stream. CCK, a satiety hormone, binds to specific receptors on the gallbladder, pancreas, stomach, and nerves to stimulate gallbladder contraction and pancreatic enzyme secretion. CCK thus has an important role in gastric emptying and satiety regulation (95). In humans, CCK secretion is stimulated by trypsin-sensitive CCK releasing factor (CCK-RF), which is produced by intestine (96). The secretion of CCK-RF is potentially degraded by trypsin; however, trypsin inhibitor from foods such as legumes may bind to trypsin, thus preventing CCK-RF degradation and stimulating CCK secretion (96). Studies have shown that oral administration of trypsin inhibitor favorably reduced energy intake both in rats (97) and human (98).

**Energy density**
Numerous studies show that food ED plays an important role in energy intake (99), satiety and weight management (100,101). The Dietary Guideline for Americans 2005 recommends a healthy eating pattern with increased consumption of fruits, vegetables, whole grains, legumes, low-fat or fat-free milk to lower energy intake and risk for chronic diseases such as obesity (102). Two food components favorably affect ED. Water, rich in many fruits and vegetables, serves the greatest impact because it contributes food amount without adding energy. Dietary fiber is another component that may lower food ED values. Legumes, rich in dietary fiber, provide 0.6-1.7 kcal/g (103). A number of human feeding studies have shown that low ED foods efficiently reduce human energy intake through satiety. Flood-Obbagy and colleagues (104) tested the effects of four preloads of apples in different forms (apple, applesauce, and apple juice with and without added fiber) on the satiety and energy intake at the follow-up lunch. They observed that eating apple and applesauce reduced lunch energy intake by 15% and 6% (P<0.0001) respectively compared to lunch consumption minus a preload. In addition, the fullness score was higher (increased fullness) for apple preload, followed by applesauce, and lastly the two juices.

Mechanisms of the cholesterol-lowering effects and the satiety effects of legumes

Short-chain fatty acid (SCFA) and cholesterol synthesis

The colonocytes digest fermented soluble fiber as their energy source, producing a large amount of SCFAs. The major SCFAs found in human colon are acetate, propionate, butyrate, valerate, hexanoate, and the branched-chain acids isobutyrate and
isovalerate. Butyrate, propionate, and acetate are predominantly taken up by the liver (105). Studies show that increases in some SCFA levels, especially butyrate, play a vital role in suppressing the biosynthesis of cholesterol (52,53). Recently, a molecular-based study evaluated the effects of SCFAs on gene expression of human enterocytes (Caco-2/TC-7 (106). The authors observed that butyrate and propionate downregulated the expression of 9 key genes involved in cholesterol biosynthesis.

Changes in plasma CCK, leptin, and ghrelin

CCK is secreted by I cells in the small intestine and regulates food intake. CCK concentration rapidly increases after food digestion in response to luminal nutrients, especially lipids and proteins, stimulating the secretion of pancreatic juices and enzymes into the duodenum and the contraction of the gallbladder for bile. CCK plays a role in satiation mainly by short-term regulation through the inhibition of gastric motility and emptying (107).

Many studies suggest that high fiber consumption causes prolonged post-meal elevated CCK concentrations. For example, in a crossover study of 10 male subjects, both a low fiber control diet and a high fiber test diet with 60g bean flakes (11.8g of dietary fiber) resulted in significantly increased CCK levels; however, the postprandial incremental CCK levels were significantly higher after the high fiber diet compared to the control (P<0.05). In addition, the CCK concentrations remained consistently high four hours after the high fiber diet, while CCK levels on the low fiber diet decreased close to the baseline level after four hours (108). In a randomized crossover study of 3 isoenergetic breakfast meals, the high-fiber low-fat diet (19% of energy from fat, 20g
dietary fiber/4.2 MJ) as well as the high-fat low-fiber diet (36% of energy from fat, 7g dietary fiber/4.2MJ) led to significant elevated post-meal CCK levels in the women (n=8), compared to the low-fiber low-fat diet (109). These studies only investigate the high fiber consumption and change in CCK after a single test meal; studies of the long-term influence of high fiber diets on changes to gut hormones are needed.

CCK may be involved in long-term food intake regulation through interplay with long-acting adiposity hormones such as leptin and ghrelin. Leptin, primarily produced by adipocytes, binds to its receptors in the hypothalamus and regulates food intake through reducing appetite and increasing energy expenditure (110). Overweight and obese persons are more likely to be leptin-resistant as well as insulin-resistant. As fat accumulates, the body compensatively increases leptin secretion in an effort to lower food intake and stimulate calorie expenditure; however, long-term exposure to elevated circulating leptin concentration may decrease tissue response to leptin administration (111). In contrast, ghrelin, a novel gut hormone primarily produced by the endocrine cells of stomach, stimulates food intake through increasing appetite and stimulating gastrointestinal motility (112). Circulating ghrelin levels rapidly rise right before and fall shortly after a meal, with involvement in the secretion of insulin, uptake of glucose and triglyceride, and lipogenesis. In addition to their roles in short-term dietary intake, there is evidence that ghrelin and leptin have roles in the long-term regulation of energy balance and body weight. Studies show that circulating leptin levels are increased and ghrelin levels are decreased in morbidly obese subjects compared to the non-morbidly obese subjects (113,114). However, with significant weight loss obese individuals experience a significant reduction in leptin and increase in ghrelin levels (113,114).
Review of clinical studies with legumes and CVD/metabolic syndrome

The beneficial effects of legume consumption on CVD and/or metabolic syndrome are widely documented. Most studies support the hypocholesterolemic and/or hypoglycemic functions of legumes. Nestel et al. (50) evaluated the dietary intakes (50 g) of either chickpeas, wheat-based foods, or white bread in 19 healthy men and women aged <70 years on three separate days. Compared to wheat-based foods or white bread consumption, chickpea consumption significantly reduced postprandial plasma glucose concentrations between 30-60 min as well as plasma insulin and HOMA-IR index at 120 min (P<0.05, respectively). Chickpea consumption, however, did not favorably reduce postprandial plasma TG compared to the other two treatments. In another chickpea feeding study by Pittaway et al. (115), 45 free-living adults with one or more CVD risk factors were assigned to a 12-week chickpea feeding period, followed by a 4-week habitual usual diet phase for metabolic changes. During the chickpea phase, subjects were instructed to incorporate a minimum of 728 g of canned, drained chickpeas per week as part of their usual diets. Both fasting serum TC and LDL-C were reduced on the chickpea diet (-7.7 and -7.3 mg/dL; P<0.01 for each) compared to their usual diets.

Several studies also evaluated the potential hypocholesterolemic and/or hypoglycemic functions of dry beans. In a randomized, controlled, cross-over study (116), 22 free living hyperlipidemic men underwent a 4-week period with their usual diets without dry beans (run-in diet). The participants were then randomly assigned to either continue their usual diets without beans or had an incorporation of 110 g/d of extruded dry beans into their usual diets over four weeks. Extruded dry bean consumption did not
significantly reduce fasting TC, LDL-C, or TG levels. In another study, Winham et al. (117) conducted a randomized, cross-over design to evaluate pinto bean consumption on the biomarkers of CVD risk. Sixteen free-living subjects aged 22-65 years with hyperinsulinemia were given three randomly ordered treatments including ½ cup of (a) pinto beans, (b) black-eyed peas, or (c) carrots (placebo) per day as part of their usual diets for 8 weeks each. Only pinto bean consumption led to fasting serum TC and LDL-C reductions which were significantly different than the other treatments (P=0.011 and 0.013, respectively). In another pinto bean feeding experiment (118) of free-living people, 40 men and women with metabolic syndrome and 40 controls were randomly assigned to either ½ cup of cooked dry pinto beans or an isocaloric chicken soup for 12 weeks as part of their usual diets. Pinto bean consumption resulted in fasting TC reductions of 4-8\% (P<0.014) and reductions in LDL-C of 7\% (P<0.05) compared to the control. The bean consumption had no effect on fasting TG or glucose. A limitation of legume-related studies is that most recent intervention studies have been conducted with a single meal or legume supplementation in free-living participants and some of studies did not track body weight change. Therefore, a feeding controlled study with weight maintenance is an important contribution to the scientific literature.

The LIFE Study provided controlled diets and carefully monitored participants’ body weight during each feeding phase. The study may supply additional knowledge on relevant effects of legume consumption on lipid profiles and satiety under conditions of weight maintenance.
References


<table>
<thead>
<tr>
<th>Type of Legumes</th>
<th>Nutrient</th>
<th>Name</th>
<th>Units</th>
<th>Value per 100 grams</th>
<th>Grams per cup</th>
<th>RDA/Al for elderly (male)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney beans, mature seeds, cooked, boiled, w/o salt</td>
<td>Fiber, total dietary</td>
<td>g</td>
<td>6.4</td>
<td>177g</td>
<td>35</td>
<td>1,200</td>
</tr>
<tr>
<td></td>
<td>Calcium, Ca</td>
<td>mg</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Magnesium, Mg</td>
<td>mg</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Potassium, K</td>
<td>mg</td>
<td>405</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sodium, Na</td>
<td>mg</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Folate, DFE</td>
<td>µg</td>
<td>130</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lima beans, thin seeded, mature seeds, cooked, boiled, w/o salt</td>
<td>Fiber, total dietary</td>
<td>g</td>
<td>7.7</td>
<td>182g</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calcium, Ca</td>
<td>mg</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Magnesium, Mg</td>
<td>mg</td>
<td>53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Potassium, K</td>
<td>mg</td>
<td>401</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sodium, Na</td>
<td>mg</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Folate, DFE</td>
<td>µg</td>
<td>150</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black beans, mature seeds, cooked, boiled, w/o salt</td>
<td>Fiber, total dietary</td>
<td>g</td>
<td>8.7</td>
<td>172g</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calcium, Ca</td>
<td>mg</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Magnesium, Mg</td>
<td>mg</td>
<td>70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Potassium, K</td>
<td>mg</td>
<td>355</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sodium, Na</td>
<td>mg</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Folate, DFE</td>
<td>µg</td>
<td>149</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Navy beans, mature seeds, cooked, boiled, w/o salt</td>
<td>Fiber, total dietary</td>
<td>g</td>
<td>10.5</td>
<td>182g</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calcium, Ca</td>
<td>mg</td>
<td>69</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Magnesium, Mg</td>
<td>mg</td>
<td>53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Potassium, K</td>
<td>mg</td>
<td>389</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sodium, Na</td>
<td>mg</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Folate, DFE</td>
<td>µg</td>
<td>140</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pinto beans, mature seeds, cooked, boiled, w/o salt</td>
<td>Fiber, total dietary</td>
<td>g</td>
<td>9.0</td>
<td>171g</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calcium, Ca</td>
<td>mg</td>
<td>46</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Magnesium, Mg</td>
<td>mg</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Potassium, K</td>
<td>mg</td>
<td>436</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sodium, Na</td>
<td>mg</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Folate, DFE</td>
<td>µg</td>
<td>172</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chapter 2

A high legume low glycemic index diet improves serum lipid profiles in men

Abstract

Background: Clinical studies have shown that healthy recommended dietary patterns improve lipid profiles; however, the beneficial effects of high fermentable fiber low glycemic index (GI) diets are unclear. Methods: The Legume Inflammation Feeding Experiment was a randomized controlled cross-over feeding study designed to evaluate the effects of such a diet on lipid profiles under conditions of weight maintenance. Sixty-four middle-aged men who had undergone colonoscopies within the previous two years were recruited in Central Pennsylvania and received both a control healthy American (HA) diet and a legume enriched, high fiber, low GI diet (LG) in random order. Diets were isocaloric and controlled for macronutrients including saturated fat; they were consumed each for four weeks with a 2-4 week break separating dietary treatments. Fasting serum samples were collected at the beginning and the end of each diet period. Results: Compared to the HA diet, the LG diet led to greater declines in both total cholesterol (TC) and LDL-C (P=0.0004 and 0.0011, respectively). The LG diet significantly decreased serum TC (10%), LDL-C (10.9%), and triglyceride (TG, 14.8%) concentrations (P<0.01 for each) after the four week feeding period. Insulin-sensitive subjects showed significant improvements in the TC/HDL-C, LDL-C/HDL-C and TG/HDL-C ratios after the LG diet; however, these ratios did not improve in insulin-resistant participants. Conclusion: A high legume, high fiber, low GI diet improves
serum lipid profiles in men. However, insulin-resistant individuals do not achieve the full benefits of the same diet on CVD lipid risk factors.
**Introduction**

Numerous studies have shown an inverse association between intake of dietary fiber and risk for chronic diseases such as cardiovascular disease CVD (1, 2) and type 2 diabetes mellitus (3). The beneficial effects may be partially due to fiber’s hypocholesterolemic (4-6) and hypoinsulinemic effects (3). Recently, the Polyp Prevention Trial, a multicenter randomized clinical trial of 1905 subjects, showed that legume consumption was significantly associated with a reduction in advanced colonic adenoma recurrence, suggesting a beneficial effect of fiber on colorectal cancer prevention (7). On the basis of a number of epidemiological studies on coronary heart disease (8-10), the Institute of Medicine (IOM) currently recommend a daily consumption of 14g of fiber per 1000 kcal for Americans (DGA, 2005).

Legumes, such as pinto, navy, and kidney beans, are a rich source of dietary fiber but data from relevant well-controlled clinical feeding studies are limited. A meta-analysis of 67 controlled trials was conducted to quantify the hypocholesterolemic effects of major dietary fibers (11). The results demonstrated that daily intake of 2-10g of soluble fiber was associated with significant decreases in total cholesterol (TC, -1.7mg/L per gram) and LDL-C (-2.2mg/L per gram). A 12-week randomized dietary intervention with 40 men and women with metabolic syndrome and 40 healthy controls (12) reported that daily intake of a bean entrée (130g of cooked dry pinto beans) lowered serum TC by 8% in the healthy population and by 4% in the metabolic syndrome group. A limitation of fiber-related studies is that most recent interventions have been conducted with a single meal (13-16) or fiber supplement (17, 18) and some of these have fairly short intervention periods (13, 15, 16).
The Legume Inflammation Feeding Experiment (LIFE Study) was a randomized controlled cross-over feeding study designed to test the effects of a legume-rich, high fermentable fiber, low glycemic index (GI) diet on biomarkers of inflammation and insulin resistance in middle-aged men at high risk for colorectal cancer. An important secondary objective of the LIFE Study was to evaluate the effects of diet on serum lipids and lipoproteins. We hypothesized that the high legume low GI diet would significantly improve serum lipid profiles. To our knowledge, this was the first randomized controlled, cross-over feeding study designed to assess the hypocholesterolemic effects of a mixture of pinto, navy, and kidney beans.

**Subjects and Methods**

**Subjects and recruitment**

All aspects of this study were approved by the Institutional Review Boards of the Pennsylvania State University and the National Cancer Institute. Sixty-six non-smoking male subjects age 35-75 who had undergone colonoscopies within the previous two years were recruited in Central Pennsylvania or by advertisements. History of colorectal adenomas (yes or no) was combined with insulin resistance (yes or no) for group classification. Insulin resistance was defined by a homeostasis model assessment (HOMA-IR) index level higher than 2.6. The formula for calculation of HOMA-IR index is as follows: fasting insulin (μU/mL) x fasting glucose (mmol/L)/22.5 (19).

**Criteria and restrictions**
All participants were prescreened by telephone interview and a clinical screening test for study eligibility. In addition to the colonoscopy, subjects had to meet the following criteria: 1) weight maintenance or less than 10% body weight loss during the past 6 months, 2) body mass index (BMI) within 20-37 kg/m², 3) no history of colorectal cancer, bowel resection or inflammatory bowel disease, 4) no serious medical conditions, e.g. heart disease, stroke, diabetes, renal or kidney disease, liver disease or cirrhosis, or cancer within the last 10 years, 5) not taking vitamin supplements, and 6) not taking any cholesterol-lowering, glucose-controlling, or non-steroidal anti-inflammatory (e.g. aspirin) medications.

The subjects who met the eligibility criteria were invited to undergo clinical screening tests including anthropometrics and a fasting blood draw at the General Clinical Research Center (GCRC) in University Park, PA. Eligible subjects were asked to return to the GCRC to have resting metabolic rate measured and then completed three 24-hour telephone diet calls (two on weekdays and one on weekend) to estimate daily energy requirements.

**Diet and study design**

A detailed explanation of diet and study design was published previously (20). The randomized controlled cross-over feeding study consisted of a first 4-week diet period, a 2-4 week compliance break, and a second 4-week diet period. The time period allowed for the test diet intervention was sufficient to assure that lipids and lipoproteins had stabilized (21). Subjects were first randomly assigned to either a healthy American diet (HA) or an isocaloric legume diet (LG; approximately 250g of cooked black, kidney,
lima, navy, and pinto beans per day), and then were switched to the other diet for the second feeding period. All foods for this study were prepared and distributed by the metabolic diet center at the GCRC. On weekdays, the participants ate breakfast or dinner at the GCRC and on Fridays, all the packed foods for weekends were taken home. The dietitians recorded body weight and food consumption at each visit. Seven-day cycle menus were created for each test diet with controlled percentages of energy contributed by each of the macronutrients. Total energy intake was adjusted by study dietitians in 200 kcal increments to maintain participants’ body weights during the diet periods. The LG diet provided much of the protein from plant sources and the HA diet provided more protein from chicken (skinless) in order to meet the dietary recommendation for cholesterol. Daily compliance questionnaires indicated very good test diet adherence. 

Table 2-1 shows the composition of the participants’ diets at entry (pre-study) and the two respective test diets.

Sample collection and measurements

At the beginning and end of each test diet period, a 12-hour overnight fasting blood sample was drawn and serum was collected for measurement of TC, LDL-C, HDL-C and TG. All blood samples were centrifuged at 3,200 xg for 15 min at 4 °C, the supernatant was separated and aliquoted in cryovials, and stored at –80 °C. Serum lipids and lipoproteins were measured at Penn State Hershey Medical Center in the laboratory of Dr. Laurence Demers. All samples from each subject were grouped together for analysis in the same batch. Lipids and lipoproteins were measured using enzymatic
procedures on an automated chemistry analyzer (ROCHE); the intra- and inter-assay coefficients of variations for all biomarkers of interest were less than 5%.

**Statistical analysis**

The experimental diets were compared with participants’ pre-study diets using one-way ANOVA with the Tukey test to adjust for the multiple comparisons. The means for the pre-study diet were calculated by 3-d diet recalls and the means for the two test diets were based on the average of each 7-d menu for a 2,000 kcal diet. Baseline characteristics stratified by subjects’ insulin resistance status were compared using one-way ANOVA. Log-transformation of TG and TG:HDL-C were performed because of skewed distributions. General linear mixed models with repeated measurement (*Proc mixed*) were used to test for the effects of diet, period, and their interactions with all outcome variables. Subject was treated as a random and diet treatment as a fixed effect. Akaike Information Criteria (AIC) and Bayesian Information criteria (BIC) were evaluated to decide model correlation structure; we used unstructured based on these comparisons and because we believed the covariance between diet treatments might be different. Likelihood ratio tests were used to test for treatment effects. Analyses were also completed using paired *t* tests with similar results. Potential confounding by age, BMI, adenoma status (yes/no), insulin resistance status (yes/no) and initial biomarker status at study entry was assessed. We also evaluated potential diet period and diet order effects for their influence on end points of interest and no carry over effect was detected. Analyses were performed using SAS version 9.1 (SAS Institute, Inc, Cary, NC). The results are reported as least square means ± SEMs. Significance was set at *P* ≤ 0.05.
Results

Sixty-four subjects finished all test diet periods. Two subjects dropped out in the first week of the first test diet period because of loss of interest (completion rate = 97%). Subjects who were insulin-resistant (IR) had higher BMIs, larger waist circumferences, and higher fasting serum glucose, insulin, and TG levels than those who were insulin-sensitive (IS) (Table 2-2). Subjects with a previous history of adenomas were older than those who were adenoma-free; other descriptive variables did not vary significantly by adenoma status (data not shown).

Effects of experimental diets on serum lipids and lipoproteins

Changes in lipids and lipoproteins on the two test diets are presented in Table 2-3. Compared with the HA diet, the LG diet resulted in significantly greater reductions in TC (-11 ± 3mg/dL, P=0.0004) and LDL-C (-9 ± 3mg/dL, P=0.0011) levels. The LG diet reduced TC (10%, P<0.0001), LDL-C (10.9%, P<0.0001), and TG (14.8%, P=0.0028) over the four week feeding period. The HA diet also reduced TC (4%, P=0.0017) and TG (12.6%, P=0.0015) but had less effect on LDL-C (3.1%, P=0.0909). Both test diets slightly lowered HDL-C concentrations. We also analyzed TC:HDL-C, LDL-C:HDL-C, and TG:HDL-C that are predictors of CVD risk. The LG diet was associated with significant reductions in TC:HDL-C (3%, P=0.0230) and LDL-C:HDL-C (3.5%, P=0.0203) but a non-significant reduction in TG:HDL-C over the four week feeding period. The HA diet was associated with non-significant reductions in TC:HDL-C and
LDL-C: HDL-C but a significant reduction in TG: HDL-C (13.5%, P=0.0262). The changes in these ratios between test diets did not differ.

**Effects of experimental diets on serum lipids and lipoproteins stratified by insulin resistance status**

We observed effect modification of the diet on biomarkers of interest by IR status (HDL-C, P-interaction=0.0293; TG, P-interaction=0.0277; TC:HDL-C, P-interaction=0.0013; TG:HDL-C, P-interaction=0.0105); therefore, we present our results stratified by insulin resistance status (IR or IS). Among IR subjects, the LG diet led to statistically significant declines in TC (8.5%, P<0.0001), HDL-C (8.9%, P<0.0001), and LDL-C (9.3%, P=0.0005) but not in TG or the HDL-C related ratios over the four week feeding period (Table 2-4). Among IS subjects, the LG diet led to statistically significant improvements in all lipid profiles and the ratios. Compared to IR subjects, IS subjects had greater reductions in TC:HDL-C (P=0.0053), LDL-C: HDL-C (P=0.0666), and TG:HDL-C (P=0.0837).

The HA diet led to significant reductions in TC (5.0%, P=0.0142), TG (24.4%, P=0.0004), TC:HDL-C (3.7%, P=0.0471), and TG:HDL-C (26.5%, P=0.0029) among IR subjects (Table 4). Among IS subjects, the HA diet resulted in significant declines in TC (3.5%, P=0.0369) and HDL-C (4.4%, P=0.0186). Compared to IR subjects, the IS subjects had smaller reductions in TG (P=0.0521) and TG:HDL-C (P=0.0406).

We calculated the differences in changes in the lipid levels and HDL-C related ratios between the two diets (change on LG diet – change on HA diet) and regressed changes on HOMA-IR values at study entry. We observed that the change in TC:HDL-C
ratio were significantly and positively correlated (smaller reductions) with the HOMA-IR value at study entry (worse insulin resistance) (β=0.266; P=0.025; Figure 2-1).

Discussion

In the LIFE study under conditions of weight maintenance, the legume-rich, high fermentable fiber, low GI diet (LG) significantly reduced fasting levels of serum lipids and lipoproteins. It is possible that the higher fiber and reduced cholesterol consumption acted to decrease fat absorption and lower hepatic synthesis of cholesterol contributing to lower circulating lipids and lipoproteins. Another possibility is that the reduced GL and GI of the legume diet played a role in favorably altering lipid concentrations. Low GI and GL diets favor insulin sensitivity. Insulin inhibits the mobilization of free fatty acids from adipose tissue, thus lowering hepatic production of very low density lipoprotein (VLDL) and maintaining low levels of TC and LDL-C. Possibly, these changes accounted for greater reductions in TC and LDL-C concentrations on the LG diet.

Many but not all (22, 23) studies have shown hypocholesterolemic effects of dietary fiber (12, 14, 17, 18, 24-26); however, studies specifically on legume consumption are still limited. Anderson et al. (4) reported that incorporating 100g of dried beans into a Western diet for 28 days decreased TC (18.7%) and LDL-C (23.1%) in men (n=6). In a feeding study of 24 hyperlipidemic men who consumed 120 g or 162 g beans with tomato sauce for 21 days (27), both serum TC and LDL-C were significantly decreased (10.4% and 8.4%, respectively). Pittaway et al. (28) reported that an addition of 104g of chickpeas into ad libitum diet for 12 weeks led to improvements in both TC (7.7mg/dL) and LDL-C (7.3mg/dL) among 13 pre- and 19 postmenopausal women and
13 men at high risk for CVD. Collectively, these studies demonstrate a hypocholesterolemic effect of dietary fiber; yet, they were not designed as controlled feeding experiments. Among these clinical studies, weight changes either were not mentioned (7, 15, and 26) or weight slightly decreased (27). In our study, subject body weights were measured daily and their calorie intake adjusted for weight maintenance; therefore, changes in lipids and lipoproteins were independent of weight loss.

The present study showed that both the LG diet and the HA diet significantly lowered fasting TG concentrations compared with study entry, which supported several (27, 29) but not all (14, 15) earlier feeding studies. We observed that the HA diet lowered fasting TG similarly to the LG diet. It is possible that the TG-lowering effects might not result from fiber consumption or GL. We then compared the total and added sugars of the two experimental diets with that of the pre-study diet; however, we did not find significant differences. The LG diet provided increased plant protein in the diet. To match protein intake and maintain a lower cholesterol intake, we added more chicken, milk, and fish to the HA diet. Differences among both test diets compared to pre-study diets (e.g. protein consumption) may have contributed to the reductions in TG observed with both diets (30).

Several cross-sectional studies have reported that high dietary GI was inversely associated with HDL-C (31-35) and positively associated with LDL-C (35) and TG concentrations (32, 35). High dietary GL was inversely associated with HDL-C (31, 32, 35, 36) and was positively associated with TG concentrations (32, 35, 36). Ma et al. (33) reported an inverse associations between GL, TC and LDL-C concentrations; however, Du et al. (34) reported non-significant associations between GL and lipid and lipoprotein
concentrations. Randomized intervention trials showed that low GL diets (37, 38) favorably improved HDL-C and/or TG concentrations. The variations of the aforementioned studies may be due to different study designs and target populations; however, unlike our study, none of these studies controlled subjects’ body weight or overall food intake.

Metabolic syndrome causes dyslipidemia partially as the result of insulin resistance. Our results demonstrate that insulin resistance may blunt response to a healthy diet. Lefevre et al. (39) reported that subjects who had higher BMIs and waist circumferences, greater percentages of body fat and higher fasting insulin concentrations had smaller reductions in TC, LDL-C, TC:HDL-C after a Step II (low fat, low saturated fat) diet. Our results are consistent with this study. We observed that subjects who had higher HOMA-IR values at the beginning of the study had smaller reductions in TG, TC:HDL-C, LDL:HDL-C, and TG:HDL-C after the LG diet, though the reduction was only statistically significant for TC:HDL-C. Different from Lefevre’s report, we found that subjects’ BMIs at study entry did not predict changes in any lipid profiles or HDL-C related ratios.

Many human clinical studies have shown that a reduced intake of saturated fat may lower the risk for cardiovascular disease by decreasing TC and LDL-C concentrations (21, 30, 39, 40). The effects of these types of diets on lowering TG levels have been variable (21, 30, 40). In the present study, we observed that moderate total (34%) and saturated fat (12%) with high fermentable fiber consumption also lowered TC and LDL-C concentrations, whereas the HA diet only lowered TC. The differences observed between test diets was significant for TC and LDL-C demonstrating an effect of
total and soluble fiber, and possibly GL. Changes of this magnitude suggest that high fermentable fiber consumption may be another approach to lower risk for CVD (41).

There are several strengths of our study. First, we implemented a randomized, cross-over controlled feeding study design. In addition, we matched the two test diets so that they were isocaloric and provided similar percentages of energy from total fat, saturated fat, carbohydrate, and protein under conditions of weight maintenance. Second, though fiber consumption was higher than daily recommendations, the LG diet was well-tolerated.

There are some limitations in the present study. The LG diet included less dietary cholesterol than the HA diet; therefore, we are not able to rule out the possibility that dietary cholesterol consumption contributed to the observed effects on serum cholesterol levels. However, our analysis showed that the difference in dietary cholesterol between the LG and HA diet was not statistically significant. In addition, a 20mg/d increment in dietary cholesterol intake only results in a very small change of serum cholesterol according to a meta-analysis of 27 studies (42). Next, we only measured the changes in selected lipid/lipoprotein biomarkers. Recent studies indicate that small LDL-C particle (sd-LDL) level is a better predictor for CHD (43) than LDL-C. Apolipoprotein B (apoB) is the primary apolipoprotein in LDL-C; measurement of apo B may provide additional information on changes in LDL particle size. Similarly, apo A-I is the primary apolipoprotein in HDL-C; measuring the change in apo A-I may help to track the change in HDL particle size.

In conclusion, this study adds to the growing evidence that incorporating legumes in the diet improves lipid profiles, thus potentially lowering CVD risk. However, a
cautionary note must be added since insulin resistance is highly prevalent and increasing, and individuals in this study with insulin resistance responded less favorably to the legume enriched, high fermentable fiber, low glycemic index diet than those without insulin resistance.
REFERENCES


Table 2-1 Nutrient and Food Profile of the Experimental Diets ( Compared to Pre-study Diet)\(^1\)

<table>
<thead>
<tr>
<th>Nutrient / Food Group</th>
<th>Pre-study Diet (Subjects’ own diet)</th>
<th>Healthy American (High GI) Diet</th>
<th>Legume (Low GI) Diet</th>
<th>P-value(^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycemic Index (^2)</td>
<td>60 ± 6</td>
<td>69 ± 3 (^4)</td>
<td>38 ± 2 (^4,5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glycemic Load (^2)</td>
<td>165 ± 77</td>
<td>152 ± 8</td>
<td>84 ± 4 (^4,5)</td>
<td>0.0049</td>
</tr>
<tr>
<td>Total Fat (% kcal)</td>
<td>35 ± 7</td>
<td>34 ± 1</td>
<td>34 ± 1</td>
<td>0.8487</td>
</tr>
<tr>
<td>SFA (% kcal)</td>
<td>12 ± 3</td>
<td>11 ± 1</td>
<td>12 ± 1</td>
<td>0.7068</td>
</tr>
<tr>
<td>Protein (% kcal)</td>
<td>16 ± 3</td>
<td>18 ± 1</td>
<td>18 ± 1</td>
<td>0.0533</td>
</tr>
<tr>
<td>Carbohydrate (% kcal)</td>
<td>48 ± 8</td>
<td>50 ± 2</td>
<td>51 ± 1</td>
<td>0.6040</td>
</tr>
<tr>
<td>Total Fiber (^2) (g/1000 kcal)</td>
<td>9 ± 3</td>
<td>9 ± 1</td>
<td>21 ± 1 (^4,5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Soluble fiber (^2)(g/1000 kcal)</td>
<td>2 ± 1</td>
<td>2 ± 1</td>
<td>4 ± 1 (^4,5)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Insoluble fiber (^2)(g/1000 kcal)</td>
<td>7 ± 2</td>
<td>7 ± 1</td>
<td>17 ± 1 (^4,5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cholesterol (^4)(mg/1000 kcal)</td>
<td>125 ± 52</td>
<td>98 ± 9</td>
<td>70 ± 11 (^4)</td>
<td>0.0098</td>
</tr>
<tr>
<td>Fruit (^2)(serving/1000 kcal)</td>
<td>0.7 ± 0.6</td>
<td>0.9 ± 0.4</td>
<td>1.3 ± 0.3 (^4)</td>
<td>0.0212</td>
</tr>
<tr>
<td>Vegetable (serving/1000 kcal)</td>
<td>1.4 ± 0.7</td>
<td>1.8 ± 0.5</td>
<td>2.2 ± 0.6 (^4)</td>
<td>0.0067</td>
</tr>
<tr>
<td>Legume (^2,3)(serving/1000 kcal)</td>
<td>0.1 ± 0.2</td>
<td>0.0 ± 0.0</td>
<td>1.5 ± 0.0 (^4,5)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\(^1\)Values are reported as mean ± S.D. The means for 3-d diet recalls were used to assess participants’ pre-study diets, the means of 7-d menus (on 2,000 kcal level) reflect the two test diets (HA and LG).
\(^2\)Data were log-transformed for analysis.
\(^3\)Legumes were excluded in the vegetables.
\(^4\)Different from pre-study diet, P<0.1.
\(^5\)Different from Healthy American diet, P<0.1.
\(^6\)P-values reflect the overall difference across the three diets using one-way ANOVA with Tukey tests to adjust for the multiple comparisons.
<table>
<thead>
<tr>
<th></th>
<th>Overall Mean</th>
<th>Insulin Resistant (IR)</th>
<th>Insulin Sensitive (IS)</th>
<th>P-value&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>64</td>
<td>28</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>54.5 ± 7.8</td>
<td>55.5 ± 8.0</td>
<td>53.8 ± 7.6</td>
<td>0.3843</td>
</tr>
<tr>
<td>BMI, kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>28.7 ± 3.5</td>
<td>30.3 ± 3.2</td>
<td>27.4 ± 3.2</td>
<td>0.0004</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>97.2 ± 9.3</td>
<td>102.3 ± 7.5</td>
<td>93.2 ± 8.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>123 ± 11</td>
<td>126 ± 12</td>
<td>121 ± 10</td>
<td>0.1122</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>81 ± 7</td>
<td>82 ± 7</td>
<td>79 ± 6</td>
<td>0.0366</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>200 ± 37</td>
<td>207 ± 37</td>
<td>195 ± 37</td>
<td>0.1887</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>45 ± 11</td>
<td>43 ± 10</td>
<td>47 ± 12</td>
<td>0.1518</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>129 ± 32</td>
<td>131 ± 35</td>
<td>127 ± 31</td>
<td>0.5986</td>
</tr>
<tr>
<td>Triglycerides&lt;sup&gt;2&lt;/sup&gt;, mg/dL</td>
<td>135 ± 72</td>
<td>171 ± 83</td>
<td>108 ± 48</td>
<td>0.0001</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>97.3 ± 8.9</td>
<td>101.9 ± 7.9</td>
<td>93.7 ± 7.9</td>
<td>0.0001</td>
</tr>
<tr>
<td>Insulin&lt;sup&gt;2&lt;/sup&gt;, μU/mL</td>
<td>11.6 ± 7.6</td>
<td>17.0 ± 8.5</td>
<td>7.4 ± 2.6</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are reported as mean ± SD.

<sup>2</sup>Data were log-transformed for the analysis.

<sup>3</sup>Baseline characteristics stratified by subject’s insulin resistance status at study entry were compared using one-way ANOVA.
Table 2-3 Effects of Diets on Lipids and Lipoproteins

<table>
<thead>
<tr>
<th></th>
<th>Baseline&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Δ LG&lt;sup&gt;4&lt;/sup&gt;</th>
<th>P-value&lt;sup&gt;5&lt;/sup&gt;</th>
<th>Δ HA&lt;sup&gt;6&lt;/sup&gt;</th>
<th>P-value&lt;sup&gt;7&lt;/sup&gt;</th>
<th>Δ LG - Δ HA&lt;sup&gt;8&lt;/sup&gt;</th>
<th>P-value&lt;sup&gt;9&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC, mg/dL</td>
<td>200 ± 5</td>
<td>-20 ± 3 (10%)</td>
<td>&lt;0.0001</td>
<td>-8 ± 3 (4%)</td>
<td>0.0017</td>
<td>-11 ± 3</td>
<td>0.0004</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>45 ± 1</td>
<td>-3 ± 1 (6.7%)</td>
<td>&lt;0.0001</td>
<td>-1 ± 1 (2.2%)</td>
<td>0.0416</td>
<td>-2 ± 1</td>
<td>0.0534</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>129 ± 4</td>
<td>-14 ± 2 (10.9%)</td>
<td>&lt;0.0001</td>
<td>-4 ± 2 (3.1%)</td>
<td>0.0909</td>
<td>-9 ± 3</td>
<td>0.0011</td>
</tr>
<tr>
<td>TG, mg/dL&lt;sup&gt;2&lt;/sup&gt;</td>
<td>135 ± 9</td>
<td>-20 ± 6 (14.8%)</td>
<td>0.0028</td>
<td>-17 ± 5 (12.6%)</td>
<td>0.0015</td>
<td>-3 ± 8</td>
<td>0.9261</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>4.61 ± 0.15</td>
<td>-0.15 ± 0.06 (3%)</td>
<td>0.0230</td>
<td>-0.08 ± 0.06 (1.7%)</td>
<td>0.1744</td>
<td>-0.07 ± 0.08</td>
<td>0.3850</td>
</tr>
<tr>
<td>LDL-C/HDL-C</td>
<td>2.97 ± 0.12</td>
<td>-0.12 ± 0.05 (3.5%)</td>
<td>0.0203</td>
<td>-0.01 ± 0.05 (0.3%)</td>
<td>0.8748</td>
<td>-0.11 ± 0.06</td>
<td>0.0866</td>
</tr>
<tr>
<td>TG/HDL-C&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3.40 ± 0.31</td>
<td>-0.28 ± 0.18 (9.4%)</td>
<td>0.2717</td>
<td>-0.40 ± 0.17 (13.5%)</td>
<td>0.0262</td>
<td>0.12 ± 0.27</td>
<td>0.5243</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are reported as mean ± S.E.M. (percentage change); n = 64. General linear mixed models with repeated measurement (Proc mixed) were used to test for the effects of diet, period, and their interactions with study outcomes.

<sup>2</sup>Data were log-transformed for the analysis.

<sup>3</sup>Baseline reflects the levels of serum lipids and lipoproteins at study entry.

<sup>4</sup>ΔLG=change over the four-week enriched-legume diet.

<sup>5</sup>Statistical significance over the enriched-legume diet.

<sup>6</sup>ΔHA=change over the four-week isocaloric healthy American diet.

<sup>7</sup>Statistical significance over the healthy American diet.

<sup>8</sup>ΔLG-ΔHA= difference in change between the two diets.

<sup>9</sup>Statistical significance of the difference in change between the two diets.
Table 2-4 Effects of Diets on Lipids and Lipoproteins among Subjects Stratified by Diet

<table>
<thead>
<tr>
<th></th>
<th>Study Entry³</th>
<th>IR⁴</th>
<th>P-value⁵</th>
<th>IS⁶</th>
<th>P-value⁷</th>
<th>IR – IS⁸</th>
<th>P-value⁹,10</th>
</tr>
</thead>
<tbody>
<tr>
<td>LG Diet (n=64)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC, mg/dL</td>
<td>200 ± 5</td>
<td>-17 ± 3 (8.5%)</td>
<td>&lt;0.0001</td>
<td>-22 ± 3 (11%)</td>
<td>&lt;0.0001</td>
<td>4 ± 5</td>
<td>0.3890</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>45 ± 1</td>
<td>-4 ± 1 (8.9%)</td>
<td>&lt;0.0001</td>
<td>-2 ± 1 (4.4%)</td>
<td>0.0150</td>
<td>-2 ± 1</td>
<td>0.1116</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>129 ± 4</td>
<td>-12 ± 3 (9.3%)</td>
<td>0.0005</td>
<td>-15 ± 3 (11.6%)</td>
<td>&lt;0.0001</td>
<td>3 ± 4</td>
<td>0.5571</td>
</tr>
<tr>
<td>TG ², mg/dL</td>
<td>135 ± 9</td>
<td>-10 ± 9 (7.4%)</td>
<td>0.2955</td>
<td>-27 ± 8 (20.0%)</td>
<td>0.0019</td>
<td>17 ± 11</td>
<td>0.1796</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>4.61 ± 0.15</td>
<td>0.05 ± 0.09 (1.1%)</td>
<td>0.5982</td>
<td>-0.30 ± 0.08 (6.5%)</td>
<td>0.0004</td>
<td>0.35 ± 0.12</td>
<td>0.0053</td>
</tr>
<tr>
<td>LDL-C/HDL-C</td>
<td>2.97 ± 0.12</td>
<td>-0.01 ± 0.07 (0.3%)</td>
<td>0.8373</td>
<td>-0.19 ± 0.06 (6.4%)</td>
<td>0.0032</td>
<td>0.18 ± 0.10</td>
<td>0.0666</td>
</tr>
<tr>
<td>TG/HDL-C ²</td>
<td>3.40 ± 0.31</td>
<td>0.21 ± 0.26 (6.2%)</td>
<td>0.5697</td>
<td>-0.67 ± 0.23 (19.7%)</td>
<td>0.0482</td>
<td>0.88 ± 0.34</td>
<td>0.0837</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Study Entry³</th>
<th>IR⁴</th>
<th>P-value⁵</th>
<th>IS⁶</th>
<th>P-value⁷</th>
<th>IR – IS⁸</th>
<th>P-value⁹,10</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA Diet (n=64)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC, mg/dL</td>
<td>200 ± 5</td>
<td>-10 ± 4 (5.0%)</td>
<td>0.0142</td>
<td>-7 ± 3 (3.5%)</td>
<td>0.0369</td>
<td>-3 ± 5</td>
<td>0.5962</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>45 ± 1</td>
<td>-0.4 ± 0.9 (0.9%)</td>
<td>0.6911</td>
<td>-2 ± 1 (4.4%)</td>
<td>0.0186</td>
<td>2 ± 1</td>
<td>0.2064</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>129 ± 4</td>
<td>-4 ± 4 (3.1%)</td>
<td>0.3264</td>
<td>-4 ± 3 (3.1%)</td>
<td>0.1681</td>
<td>1 ± 5</td>
<td>0.8794</td>
</tr>
<tr>
<td>TG ², mg/dL</td>
<td>135 ± 9</td>
<td>-33 ± 7 (24.4%)</td>
<td>0.0004</td>
<td>-5 ± 6 (3.7%)</td>
<td>0.2180</td>
<td>-28 ± 10</td>
<td>0.0521</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>4.61 ± 0.15</td>
<td>-0.17 ± 0.09 (3.7%)</td>
<td>0.0471</td>
<td>-0.01 ± 0.07 (0.2%)</td>
<td>0.9316</td>
<td>-0.17 ± 0.11</td>
<td>0.1434</td>
</tr>
<tr>
<td>LDL-C/HDL-C</td>
<td>2.97 ± 0.12</td>
<td>-0.02 ± 0.08 (0.7%)</td>
<td>0.7728</td>
<td>0.001 ± 0.069 (0.03%)</td>
<td>0.9841</td>
<td>-0.02 ± 0.11</td>
<td>0.8165</td>
</tr>
<tr>
<td>TG/HDL-C ²</td>
<td>3.40 ± 0.31</td>
<td>-0.90 ± 0.26 (26.5%)</td>
<td>0.0029</td>
<td>-0.02 ± 0.22 (0.6%)</td>
<td>0.7129</td>
<td>-0.89 ± 0.34</td>
<td>0.0406</td>
</tr>
</tbody>
</table>

¹ Values are reported as mean ± S.E.M. (percentage change). LG Diet=the enriched-legume diet. HA Diet=the healthy American diet. General linear mixed models with repeated measurement (Proc mixed) were used to test for the effects of diet, period, and their interactions with study outcomes.
Data were log-transformed for the analysis.

The levels of serum lipids and lipoproteins at study entry.

IR = changes among insulin-resistant subjects over the four-week diet treatment.

Statistical significance of the changes among insulin-resistant subjects.

IS = changes among insulin-sensitive subjects over the four-week diet treatment.

Statistical significance of the changes among insulin-sensitive subjects.

IR-IS = difference in changes between IR and IS subjects.

Statistical significance of the difference in change between IR and IS subjects.

P-values for interaction terms were not statistically significant except for P=0.0277 for HDL-C, P=0.0013 for TG, P=0.0105 for TC:HDL-C, and P=0.0105 for TG:HDL-C ratios.
Figure 2-1. Relationship between HOMA-IR index at study entry and differences in TC:HDL cholesterol ratio changes (Delta TC:HDL-C ratio) on the two diets. Delta TC:HDL-C = change in TC:HDL-C ratio on LG diet – change in TC:HDL-C ratio on HA diet (n=64; β=0.266, P=0.025).
Chapter 3

The Effects of a High Legume Low Glycemic Index Diet on Fasting Plasma Leptin and Ghrelin in Older Men

Abstract

Context: Leptin and ghrelin have been shown to play vital roles in long-term weight management. Few human feeding studies have reported the effects of diets different in macronutrient composition on changes in long-term fasting leptin and ghrelin. Specifically, the effect of a diet high in fermentable fiber from legumes on these hormones is unknown. Objective: The objective was to assess the influence of consuming a legume-enriched low glycemic index (GI) diet (four weeks) on fasting plasma leptin and ghrelin under conditions of weight maintenance. Design: Sixty-four men (age, 54.5 ± 7.8 yr; body mass index 28.7 ± 3.5 kg/m²; 36 insulin-sensitive (IS) and 28 insulin-resistant (IR)) consumed the legume-enriched low GI diet (LG) (approximately 1 ½ cups of cooked navy, pinto, and kidney beans per 2,000 kcal) and an isocaloric healthy American diet (HA) each for four weeks in random order separated by a 2-week compliance break. Energy intakes were adjusted for weight maintenance across the study.

Main Outcome Measures: We measured over-night fasting plasma leptin and ghrelin at the beginning and end of each diet treatment. Results: Both diets significantly reduced fasting leptin concentration with no statistically significant between-diet differences.
Neither dietary treatment significantly altered fasting ghrelin concentration. Both the IR and IS subjects showed significant reductions in fasting leptin after the LG diet; the IR subjects also showed significant improvements in leptin after the HA diet. **Conclusions:** Both two test diets efficaciously lowered fasting plasma leptin even with weight maintenance. Insulin resistance status may be an effect modifier for the effect of diet on leptin response.
Introduction

Leptin, a 16 kDa protein encoded by the *ob* gene, is produced primarily by the adipose tissues and is thought to play a key role in weight maintenance. In a well-known animal experiment, leptin administration to leptin-deficient *ob/ob* mice led to reduced food intake, weight loss, and increased energy expenditure (1, 2). Leptin binds to its receptors in the hypothalamus and functions partially by inhibiting the activity of neuropeptide Y (NPY) containing neurons (feeding stimulators) and increasing the activity of neurons expressing α-melanocyte-stimulating hormone (satiety mediators) (3).

Ghrelin, a 28-amino acid peptide, is a novel gut hormone primarily produced by the endocrine cells of the stomach. Ghrelin acts as an endogenous ligand for the growth hormone (GH) secretagogue receptor (4). Ghrelin administration stimulates food intake in humans through increasing appetite and gastrointestinal motility (5). Similar to leptin, ghrelin also has a role in the NPY receptor pathway; however, ghrelin is a potent NYP activator (6), suggesting that ghrelin may counteract leptin’s effects. Circulating ghrelin concentrations rapidly rise right before and fall shortly after a meal in response to insulin secretion (7, 8).

Leptin and ghrelin are involved in long-term weight regulation. Patients with obesity, insulin resistance, and type 2 diabetes have higher circulating leptin and lower ghrelin concentrations (9-11). In contrast, weight loss decreases leptin concentrations and increases ghrelin concentrations (12-14).

Epidemiological and clinical studies have shown that dietary fiber is associated with a lower risk for heart disease (15, 16), type 2 diabetes (17), and obesity (18-20). Soluble fiber, high in oat bran, legumes, and barley, etc., becomes viscous during
digestion, delays gastric emptying, and increases satiety. Metabolic studies suggest that dietary fiber, especially soluble fiber, may contribute to postprandial satiety by contributing to changes in satiety hormones such as leptin and ghrelin (21). Dietary intervention studies in humans show that high dietary fiber consumption is related to weight loss (22), supporting the potential influence of fiber on circulating leptin and ghrelin concentrations. To date, few human feeding studies but some animal studies have been conducted to evaluate the long-term effect of legume consumption on these hormones. In a 12-week fiber-enriched intervention study with high-fat diet alone (HFD), high-fat diet with 10% sugarcane fiber (SCF), 10% psyllium (PSY), or 10% cellulose (CEL), fasting leptin concentrations were significantly lower among the C57BL/6 mice in the SCF and PSY groups than those in the CEL and HFD groups. In addition, stomach ghrelin mRNA levels in the SCF were significantly lower than other groups, followed by the PSY and CEL groups (23). Maurer et al. reported that a 1- and 2-week high-fiber weaning diet (25% wt/wt) resulted in a significant leptin reduction in virgin female Wistar rats, compared to the high-protein weaning diet or the control (24).

The Legume Inflammation Feeding Experiment (LIFE Study) was a randomized controlled cross-over feeding study designed to determine the effects of a legume-enriched low glycemic index (GI) diet on the biomarkers of inflammation and insulin resistance in men at high risk for colorectal cancer. An important secondary objective of the LIFE Study was to evaluate the effects of the diet on fasting plasma leptin and ghrelin. To our knowledge, this was the first randomized controlled cross-over feeding study to evaluate the long-term effects of legumes, such as pinto, navy, and kidney beans, on changes in fasting leptin and ghrelin levels.
Subjects and Methods

Subjects and recruitment

Sixty-six non-smoking men were recruited from Central Pennsylvania. All subjects were between 35-75 years of age and had undergone colonoscopies within the previous two years. Insulin sensitivity status was ascertained by homeostasis model assessment (HOMA) index level. The HOMA index was calculated as follows: fasting serum insulin (μU/mL) x fasting glucose (mmol/L)/22.5 (25). Subjects were defined as insulin-resistant if their HOMA index values were higher than 2.6. Subjects were recruited to encompass the spectrum of insulin sensitivity/resistance. All aspects of this study were approved by the Institutional Review Boards of the Pennsylvania State University and the National Cancer Institute.

Criteria and restrictions

Details of the inclusion and exclusion criteria as well as of the experiment itself have been previously reported (26). Briefly, all participants were prescreened by telephone interview. Qualified subjects had BMI values between 20-37 kg/m² and had no history of colorectal cancer, inflammatory bowel disease, heart disease, stroke, diabetes, renal or kidney disease, liver disease or cirrhosis, or other cancer with the exception of benign skin cancer. In addition, subjects could not be currently using vitamin supplements, cholesterol-lowering medications, non-steroidal anti-inflammatory drugs, or
glucose-controlling medications, and could not have lost ≥ 10% of their body weight over the previous 6 months.

The subjects who met the requirements during the telephone interview were invited to undergo clinical screening at the Penn State University General Clinical Research Center (GCRC), which included the measurement of height, weight, waist circumference and blood pressure, and an overnight fasting blood draw. Eligible subjects returned to the GCRC to have a resting metabolic rate (RMR) test to estimate energy requirements. Three random 24-hour telephone diet recalls (two weekdays and one weekend) were collected by study dietitians to serve as an additional guide for energy intake, as well as providing estimates of pre-study macro- and micronutrient intakes.

**Diet intervention and study design**

The diet intervention was designed as a randomized controlled cross-over feeding study with a first 4-week diet period, a 2-week washout period, and a second 4-week diet period. Subjects received both a legume-enriched diet (LG; approximately 1 ½ cups of cooked pinto, navy, and kidney beans/2,000 kcal) and an isocaloric healthy American diet (HA) in random order. All foods were provided by the metabolic dietary center at the GCRC. The nutrient profiles for the respective diets are shown in Table 3-1. Study dietitians weighed the subjects and recorded food consumption daily. Calorie intake was adjusted by the dietitians in order to maintain participants’ body weights throughout the two diet periods. On weekdays, the participants consumed either breakfast or dinner at the GCRC and the other meals were packed in coolers for the subjects to take with them.
All the foods for weekends were taken home by the participants on Fridays. During the 2-week washout, the dietary center did not provide foods and the participants ate ad libitum.

**Sample collection and measurements**

Twelve-hour overnight fasting blood samples were collected at the beginning and end of each dietary period for the measurement of leptin and ghrelin. All blood samples were centrifuged at 3,200 xg for 15 min at 4 °C, supernatant was separated and aliquoted into cryovials, and stored at –80 °C. Hormone concentrations were measured in the laboratory of Dr. A. Catharine Ross, Penn State University. All of each subject’s samples were grouped together for analysis in the same batch. Plasma leptin was measured using a human leptin ELISA (Linco, MA, EZHL-80SK; sensitivity, 0.5-100 ng/mL; within-assay CV, 3.7%; between-assay CV, 4%). Plasma ghrelin was measured using ghrelin (total) RIA (Linco, MA, GHRT-89HK; sensitivity, 93 pg/mL; within-assay CV, 6.4%; between-assay CV, 16.3%; spike and recovery in human plasma, 92.3%). A detailed description of the procedures used for the measurement of glucose, insulin, and C-peptide were presented in our earlier report (26).

**Statistical analysis**

The experimental diets were compared with the prestudy diet and to each other using one-way ANOVA. Tukey test was conducted for pairwise comparisons. Baseline characteristics stratified by subjects’ insulin resistance status were compared using one-way ANOVA. Leptin values were log-transformed to normalize the distribution. Linear mixed models were used to test for the effects of diet, period, and their interactions for all
continuous outcomes. Linear mixed models were fit to the data using *Proc Mixed* in SAS. The likelihood ratio test was used to test for treatment effects. The analyses were also completed using *t* test with similar results. Potential confounding by age, baseline BMI, polyp status, and initial biomarker status was assessed. Our results indicated that insulin resistant (IR) status may be an important consideration; therefore, the analyses were also conducted by IR stratification and the interaction of diet and IR status was tested. However, there was no evidence of this for polyp status. Pearson correlations and linear regressions were conducted to evaluate the associations between the changes in the gut hormones and the changes in the markers of insulin resistance. Calculations were performed using SAS version 9.1 (SAS Institute, Inc, Cary, NC). The results are reported as least square means ± SEMs. Significance was set at *P* ≤ 0.05.

**Results**

**Baseline characteristics**

Sixty-four subjects finished all diet periods and two subjects dropped out in the first week of the first diet period (completion rate = 97%). Subjects’ baseline characteristics are summarized in Table 3-2. In brief, insulin-resistant (IR+) subjects had higher BMIs (*P*=0.0004), fasting glucose (*P*=0.0001), insulin (*P*<0.0001), C-peptide (*P*<0.0001), and leptin (*P*<0.0001) and lower ghrelin (*P*=0.0188) levels than those who were insulin-sensitive (IR-). At baseline, fasting leptin concentration was inversely associated with ghrelin (*r*=-0.551, *P*<0.0001) and positively associated with BMI (*r*=0.762, *P*<0.0001), glucose (*r*=0.273, *P*=0.0288), insulin (*r*=0.533, *P*<0.0001), and C-peptide (*r*=0.689, *P*<0.0001). Fasting ghrelin concentration was inversely associated with
BMI (r=-0.568, P<0.0001), insulin (r=-0.468, P<0.0001), and C-peptide (r=-0.433, P=0.0004) (data not shown).

Effects of experimental diets on fasting leptin and ghrelin

The diet-related changes in fasting leptin and ghrelin levels are shown in Table 3. Both the LG and the HA diets resulted in significant reductions on fasting leptin (18.8%, P<0.0001 and 16.1%, P=0.0003, respectively). The LG diet slightly decreased (2.3%) and the HA diet increased (2.9%) ghrelin concentrations (NS). There were no statistically significant differences between effects of the diets for either hormone.

Effects of experimental diets on fasting leptin and ghrelin stratified by insulin sensitivity status

The changes in fasting leptin and ghrelin among IR and IS subjects are shown in Table 3-4. The LG diet led to significant reductions in fasting leptin concentration among both IR and IS subjects (IR, 24.4%, P=0.0085; IS, 15.4%, P=0.0009); the reductions between IR and IS subjects were not statistically significant. The HA diet led to a significant reduction in fasting leptin concentration among IR subjects (33.3%, P<0.0001), but the reduction among IS subjects was not statistically significant (2.6%, NS). Compared to IS subjects, IR subjects showed a greater reduction in fasting leptin concentration (P=0.0094) after the HA diet.

For fasting ghrelin, the LG diet resulted in a greater reduction among IS subjects than that of among IR subjects (3.9% vs 0.3%, NS). In contrast, the HA diet resulted in a greater increase among IR subjects than that of among IS subjects (6.4% vs 0.1%, NS).
The changes of fasting ghrelin between IR and IS subjects were not statistically significant regardless of the two diet treatments.

**Associations among the changes in study variables**

Detailed information on the changes in fasting glucose, insulin, and C-peptide were presented in our earlier paper (26). Overall, the change in fasting leptin level was positively correlated with the change in fasting glucose (r=0.204, P=0.0251), insulin (r=0.447, P<0.0001), and C-peptide (r=0.492, P<0.0001) levels. There was an inverse correlation trend between the changes in leptin and ghrelin levels (r=-0.161, NS).

The observed correlations were dependent on dietary treatment. For the LG diet, there were positively associations between the change in fasting leptin level and the changes in fasting glucose (β=0.11, P=0.0231; **Figure 3-1**), insulin (β=0.47, P<0.0001; **Figure 3-2**), and C-peptide levels (β=2.52, P<0.0001; **Figure 3-3**). Additionally, there was a negative correlation between the changes in fasting ghrelin and C-peptide levels (β=-57, P=0.0285; **Figure 3-4**). For the HA diet, there were positive associations between the change in fasting leptin and the changes in fasting insulin (β=0.18, P=0.0005; **Figure 3-6**) and C-peptide concentrations (β=1.52, P=0.0054; **Figure 3-7**), but not for glucose (β=0.08, P=0.0814; **Figure 3-5**). All the ghrelin-related correlations were, however, not significant for the HA diet (data not shown).

**Discussion**

In the LIFE study, we observed that subjects’ baseline fasting leptin and ghrelin concentrations were highly related to insulin sensitivity status: IR subjects had higher
leptin but lower ghrelin levels than IS subjects. Our results supported the evidence found in many cross-sectional studies (10, 27-30). Similarly we have confirmed that obese people have higher fasting leptin and lower ghrelin levels compared to people of normal weight. Leptin is primarily secreted from adipose tissues to reduce food intake and increase energy expenditure (1, 2). Similar to the response of insulin with insulin resistance, circulating leptin compensatorily elevates, which leads to leptin resistance. Ghrelin is a “hunger” hormone; metabolic studies have shown that insulin and leptin suppress ghrelin production (31-33); therefore, the fasting ghrelin concentrations may decrease under conditions of hyperinsulinemia and hyperleptinemia as we have observed in this study.

In the present study, we observed that both the LG and the HA diets favorably decreased fasting leptin concentrations. We compared the differences of the major nutrients and food profiles of the subjects’ own diets (pre-study diet), LG diet, and HA diet (Table 3-1) and observed that both diets were more healthful than the subjects’ pre-study diets. The fruit and vegetables included with the LG and HA diets were both higher of the subjects’ pre-study diet. The LG diet contained 1.3 servings / 1,000 kcal of fruit and 2.2 servings / 1,000 kcal of vegetables, HA diet contained 0.9 serving / 1,000 kcal of fruit and 1.8 servings / 1,000 kcal of vegetables, whereas the pre-study only contained 0.7 serving / 1,000 kcal of fruit and 1.4 servings / 1,000 kcal of vegetables. The pre-study diet also contained higher dietary cholesterol (125 mg/1,000 kcal) than the LG and HA diets, possibly, our subjects ate more red meat and processed meat in their typical diet, and when they were on the test diets their fasting leptin concentrations were improved.
Additionally, we also observed that insulin sensitivity status was influential in subjects’ response to the LG and the HA diets. For example, among IS subjects, the LG diet led to significantly reduced fasting leptin levels (22%), whereas the HA diet only slightly decreased leptin levels (4.6%). Among IR subjects, both diets significantly reduced leptin levels. We compared the major nutrients and food profiles of the pre-study diet by subjects’ insulin sensitivity status to evaluate potential differences in diet but did not observe any statistical differences. For instance, the fruit and vegetable consumptions as well as the glycemic indices and glycemic loads of the diets among IR subjects were similar to those among IS subjects. Therefore, we cannot explain the reasons why IR and IS subjects had different responses to the test diets. Perhaps, this might partially be due to metabolic alterations among IR subjects.

The LIFE study showed that the LG diet led to reduced fasting ghrelin concentrations whereas the HA diet resulted in elevated ghrelin concentrations. As mentioned earlier, ghrelin, an orexigenic hormone, stimulates appetite and initiates food intake, since ghrelin antagonizes leptin on the activity of NPY (3, 6). However, the physiological regulation of ghrelin remains uncertain. Rodent research found that after consuming a high-fat diet, the obesity-prone rats gained body weight and rapidly developed hyperleptinemia accompanied with ghrelin suppression, whereas the obesity-resistant rats maintained their body weight and the secretions of leptin and ghrelin were unchanged. This evidence indicates that hyperleptinemia may inhibit ghrelin production (34). Our results did not support this hypothesis because fasting ghrelin concentrations decreased after 4-week LG diet intervention. One possible explanation may be that the high legume consumption influenced hormone regulation. Another explanation to the
discrepancy is that the different functions of acylated and desacylated ghrelin. Acylated ghrelin has n-octanoic or other medium-chain fatty acids at the serine 3 position and is believed to act as the active form of ghrelin; on the other hand, desacylated ghrelin lacks the n-octanoic residue and is concerned to antagonize acylated ghrelin (35-37). Molecular studies also indicate that the acylated:desacylated ghrelin ratio may affect ghrelin physiological function (38). In the present study, we only measured total ghrelin (acylated and desacylated ghrelin) but not the active form of ghrelin.

We observed that decreased leptin concentration was significantly correlated with decreased fasting insulin and C-peptide concentrations; in addition, the correlations during the LG diet were stronger than that during HA diet. Several molecular studies demonstrate that insulin stimulates ob gene expression and chronically increases leptin production both in vivo and in vitro (39-42). In this study, both the LG and the HA diets led to slight but non-significant reductions in fasting insulin (26); possibly, the slight reductions of insulin concentrations were enough to suppress leptin production. C-peptide derives from proinsulin, which is cleaved into equal molecules of C-peptide and insulin. Several studies demonstrate that C-peptide is an independent indicator for insulin status since the peripheral clearance of C-peptide is much longer than insulin itself. In the present study, we observed that both the changes in fasting insulin and C-peptide had similar correlations with the changes in fasting leptin.

A major strength of the present study is that we maintained subjects’ body weight to rule out the influence of weight loss on hormone concentrations. A number of studies show that weight loss may directly change fasting leptin and ghrelin levels, independent of nutrition-induced regimens (14, 43, 44). In the present study, we monitored subjects’
daily body weight and adjusted energy intake to maintain the subjects’ weight during each 4-week feeding period. Additionally, the randomized cross-over design of the present study minimized individual variation and maximized statistical power, since each participant served as his own control.

This study has some limitations. For example, we only measured total ghrelin level. As mentioned earlier, acylated ghrelin (active ghrelin) may play a vital role in meal initiation while desacylated ghrelin may have opposite functions. Without the information on acylated ghrelin, we may not clearly interpret the change of ghrelin level during the diet intervention.

In conclusion, our findings demonstrate that 40 g of dietary fiber consumption from the mixture of 1 ½ cups of navy, pinto, and kidney beans efficaciously lowered fasting leptin levels with weight maintenance but had no effect on fasting ghrelin concentrations. Insulin sensitivity status played a role in the nutrition-induced response; the insulin-sensitive subjects only responded favorably to the high legume diet; whereas the insulin-resistant subjects responded favorably to both test diets.
References


Charro AL 2008 Effects of weight loss after bariatric surgery for morbid obesity on vascular endothelial growth factor-A, adipocytokines, and insulin. J Clin Endocrinol Metab 93:4276-4281


Resistance and Inflammation among Men at Risk for Colorectal Cancer. J Nutr 140:60-67

27. **Purnell JQ, Weigle DS, Breen P, Cummings DE** 2003 Ghrelin levels correlate with insulin levels, insulin resistance, and high-density lipoprotein cholesterol, but not with gender, menopausal status, or cortisol levels in humans. J Clin Endocrinol Metab 88:5747-5752


38. **Thompson NM, Gill DA, Davies R, Loveridge N, Houston PA, Robinson IC, Wells T** 2004 Ghrelin and des-octanoyl ghrelin promote adipogenesis directly in vivo by a mechanism independent of the type 1a growth hormone secretagogue receptor. Endocrinology 145:234-242


Table 3-1: Nutrient and Food Profile of the Experimental Diets (Compared to Pre-study Diet) 1

<table>
<thead>
<tr>
<th>Nutrient / Food Group</th>
<th>Pre-study Diet (Subjects' own diet)</th>
<th>Healthy American (High GI) Diet</th>
<th>Legume (Low GI) Diet</th>
<th>P-value 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycemic Index 2</td>
<td>60 ± 6</td>
<td>69 ± 3 3</td>
<td>38 ± 2 4,5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glycemic Load 2</td>
<td>165 ± 77</td>
<td>152 ± 8</td>
<td>84 ± 4 4,5</td>
<td>0.0049</td>
</tr>
<tr>
<td>Total Fat (% kcal)</td>
<td>35 ± 7</td>
<td>34 ± 1</td>
<td>34 ± 1</td>
<td>0.8487</td>
</tr>
<tr>
<td>SFA (% kcal)</td>
<td>12 ± 3</td>
<td>11 ± 1</td>
<td>12 ± 1</td>
<td>0.7068</td>
</tr>
<tr>
<td>Protein (% kcal)</td>
<td>16 ± 3</td>
<td>18 ± 1</td>
<td>18 ± 1</td>
<td>0.0533</td>
</tr>
<tr>
<td>Carbohydrate (% kcal)</td>
<td>48 ± 8</td>
<td>50 ± 2</td>
<td>51 ± 1</td>
<td>0.6040</td>
</tr>
<tr>
<td>Total Fiber 2 (g/1000 kcal)</td>
<td>9 ± 3</td>
<td>9 ± 1</td>
<td>21 ± 1 4,5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cholesterol 2 (mg/1000 kcal)</td>
<td>123 ± 52</td>
<td>98 ± 9</td>
<td>70 ± 11 4</td>
<td>0.0098</td>
</tr>
<tr>
<td>Fruit 2 (serving/ 1000 kcal)</td>
<td>0.7 ± 0.6</td>
<td>0.9 ± 0.4</td>
<td>1.3 ± 0.3 4</td>
<td>0.0212</td>
</tr>
<tr>
<td>Vegetable (serving/ 1000 kcal)</td>
<td>1.4 ± 0.7</td>
<td>1.8 ± 0.5</td>
<td>2.2 ± 0.6 4</td>
<td>0.0067</td>
</tr>
<tr>
<td>Legume 2,3 (serving/ 1000 kcal)</td>
<td>0.1 ± 0.2</td>
<td>0.0 ± 0.0</td>
<td>1.5 ± 0.0 4,5</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

1 Values are reported as mean ± S.D.; n=64.
2 Data were log-transformed for the analysis.
3 Legumes were excluded in the vegetables.
4 Different from pre-study diet, P<0.1.
5 Different from Healthy American diet, P<0.1.
6 One-way ANOVA was used and Tukey method was used for the pairwise comparisons.
Table 3-2 Baseline Characteristics of Study Subjects

<table>
<thead>
<tr>
<th></th>
<th>Overall Mean</th>
<th>Insulin Resistant (IR)</th>
<th>Insulin Sensitive (IS)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>64</td>
<td>28</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>54.5 ± 7.8</td>
<td>55.5 ± 8.0</td>
<td>53.8 ± 7.6</td>
<td>0.3843</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.7 ± 3.5</td>
<td>30.3 ± 3.2</td>
<td>27.4 ± 3.2</td>
<td>0.0004</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>97.3 ± 8.9</td>
<td>101.9 ± 7.9</td>
<td>93.7 ± 7.9</td>
<td>0.0001</td>
</tr>
<tr>
<td>Insulin, μU/mL</td>
<td>11.6 ± 7.6</td>
<td>17.0 ± 8.5</td>
<td>7.4 ± 2.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C-peptide, ng/mL</td>
<td>2.3 ± 1.1</td>
<td>3.1 ± 1.2</td>
<td>1.7 ± 0.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Leptin ², ng/mL</td>
<td>7.8 ± 6.1</td>
<td>11.1 ± 6.1</td>
<td>5.2 ± 4.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ghrelin (total), pg/mL</td>
<td>670 ± 281</td>
<td>578 ± 277</td>
<td>742 ± 266</td>
<td>0.0188</td>
</tr>
</tbody>
</table>

¹ Values are reported as mean ± SD.
² Data were log-transformed for the analysis.
³ IR = baseline characteristics of insulin resistant subjects.
⁴ IS = baseline characteristics of insulin sensitive subjects.
⁵ One-way ANOVA was used for the comparisons.
Table 3-3 Effects of Diets on Leptin and Ghrelin

<table>
<thead>
<tr>
<th></th>
<th>Study Entry $^3$</th>
<th>Δ LG $^4$</th>
<th>P-value</th>
<th>Δ HA $^5$</th>
<th>P-value</th>
<th>Δ LG-Δ HA $^6$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin $^1$, ng/mL</td>
<td>7.8 ± 6.1</td>
<td>-1.5 ± 0.3 (18.8%)</td>
<td>&lt;0.0001</td>
<td>-1.3 ± 0.3 (16.1%)</td>
<td>0.0003</td>
<td>-0.2 ± 0.4</td>
<td>0.1499</td>
</tr>
<tr>
<td>Ghrelin, pg/mL</td>
<td>670 ± 281</td>
<td>-15 ± 17 (2.3%)</td>
<td>0.3637</td>
<td>19 ± 20 (2.9%)</td>
<td>0.3330</td>
<td>-34 ± 23</td>
<td>0.1381</td>
</tr>
</tbody>
</table>

$^1$ Values are reported as mean ± S.E.M.; n = 64.
$^2$ Data were log-transformed for the analysis.
$^3$ The levels of fasting plasma leptin and ghrelin at study entry.
$^4$ ΔLG = change over the legume-enriched diet.
$^5$ ΔHA = change over the isocaloric healthy American diet.
$^6$ ΔLG-ΔHA = change between the two diets.
### Table 3-4 Effects of Diets on Leptin and Ghrelin among Subjects Stratified by Diet

<table>
<thead>
<tr>
<th>Diet</th>
<th>Study Entry</th>
<th>IR</th>
<th>P-value</th>
<th>IS</th>
<th>P-value</th>
<th>IR-IS</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LG Diet (n=64)</td>
<td>7.8 ± 6.1</td>
<td>-1.9 ± 0.5 (24.4%)</td>
<td>0.0085</td>
<td>-1.2 ± 0.5 (15.4%)</td>
<td>0.0009</td>
<td>-0.7 ± 0.7</td>
<td>0.7593</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
<td>670 ± 281</td>
<td>-2 ± 25 (0.3%)</td>
<td>0.9506</td>
<td>-26 ± 22 (3.9%)</td>
<td>0.2524</td>
<td>24 ± 34</td>
<td>0.4765</td>
</tr>
<tr>
<td>HA Diet (n=64)</td>
<td>7.8 ± 6.1</td>
<td>-2.6 ± 0.4 (33.3%)</td>
<td>&lt;0.0001</td>
<td>-0.2 ± 0.4 (2.6%)</td>
<td>0.2150</td>
<td>-2.3 ± 0.6</td>
<td>0.0094</td>
</tr>
<tr>
<td>Leptin, pg/mL</td>
<td>670 ± 281</td>
<td>43 ± 30 (6.4%)</td>
<td>0.1540</td>
<td>1 ± 26 (0.1%)</td>
<td>0.9809</td>
<td>42 ± 40</td>
<td>0.2903</td>
</tr>
</tbody>
</table>

1. Values are reported as mean ± S.E.M.
2. Data were log-transformed for the analysis.
3. The levels of fasting plasma leptin and ghrelin at study entry.
4. IR=changes among insulin-resistant subjects.
5. IS=changes among insulin-sensitive subjects.
6. IR-IS=changes between IR and IS subjects.
Figure 3-1 Positive association between changes in fasting glucose and changes in fasting leptin during the LG diet (n=64; β=0.11; P=0.0231).
Figure 3-2 Positive association between changes in fasting insulin and changes in fasting leptin during the LG diet (n=64; β=0.47; P<0.0001).
Figure 3-3 Positive association between changes in fasting C-peptide and changes in fasting leptin during the LG diet (n=64; β=2.52; P<0.0001).
Figure 3-4 Negative association between changes in fasting C-peptide and changes in fasting ghrelin during the LG diet (n=64; β=-.57; P=0.0285).
Figure 3.5 Positive association between changes in fasting glucose and changes in fasting leptin during the HA diet (n=64; β=0.08; P=0.0814).
Figure 3-6 Positive association between changes in fasting insulin and changes in fasting leptin during the HA diet (n=64; β=0.18; P=0.0005).
Figure 3-7 Positive association between changes in fasting C-peptide and changes in fasting leptin during the HA diet (n=64; β=1.52; P=0.0054).
Chapter 4
Summary, Strengths, Limitations and Future Directions

Summary

The present study is the first human controlled feeding study with high legume consumption (~1 ½ cups of cook dry beans), a low glycemic index (38) and low energy density (1.15) diet. Our study clearly demonstrates the benefits of this dietary regimen under weight stable conditions on risk factors for metabolic syndrome and CVD. The 4-week diet intervention significantly reduced TC (-20 mg/dL; 10%), LDL-C (-14 mg/dL; ~11%), and TG (-20 mg/dL; ~15%) concentrations as well as TC:HDL-C (-0.15) and LDL-C:HDL-C (-0.12) ratios. Our study control, the HA diet, also had beneficial effects on some lipid profiles but not on LDL-C. Our results also demonstrate that insulin resistance blunts the cholesterol-lowering benefits of high legume consumption. We observed that the LG diet favorably improved all lipids, most lipoproteins (not HDL-C), and their ratios among insulin-sensitive subjects; however, significant reductions were only observed in TC and LDL-C levels among IR subjects.

We observed that the legume intervention significantly lowered fasting plasma leptin concentrations (-1.5 ng/mL; ~19%) and only slightly decreased fasting plasma ghrelin concentrations (-15 pg/mL, ~2%). Leptin concentrations substantially decreased among all subjects. The changes in ghrelin concentrations were not different between IS or IR subjects.

In the previously published results(1), we also observed that the LG diet led to significant reductions on biomarkers of inflammation. The intervention had no effects on
changes in fasting insulin and C-peptide levels but surprisingly increased fasting glucose levels. The HA diet had similar effects on the changes in the aforementioned markers except that it resulted in significant reductions in fasting glucose levels. The previous published study also reported that insulin resistance status may blunt the beneficial effects of healthful dietary change.

Collectively, the LG diet may influence satiety and ameliorate inflammation, CVD risk (lipids and lipoproteins) but had no effects on insulin resistance. Perhaps, a 4-week dietary intervention may not be long enough to completely shift body metabolism or make a change. Moreover, all subjects were asked to maintain their levels of physical activity; possibly, increase physical activity with healthful dietary intervention may favorably improve insulin sensitivity than dietary intervention alone (2-4). During the 4-week optional weight loss phase, subjects (n=44) were counseled to consume all legume portions in each meal but have other foods provided as desired. We observed that subjects lost an average of 4 kg of body weight (4.4%, P<0.01), and all aforementioned biomarkers were significantly improved (data not shown). Our results suggested that legume-enriched dietary regimen may facilitate weight loss and the synergism may be more efficacious than dietary intervention alone.

In the LIFE Study, we observed that both the HA and LG diets had favorable effects on many study endpoints. Generally, both the HA and LG diets tended to be more healthful than subjects’ prestudy diets. We compared GI, GL, and important nutrients and food groups for the subjects’ usual diets and the HA and LG test diets. Although some of the differences were not significant, both of the two test diets had higher concentrations of total carotenoids, vitamin D, vitamin E, vitamin C, folate, fruits and vegetables but
lower cholesterol compared to usual dietary intakes (1). Epidemiological and clinical studies show an inverse association between fruit and vegetable consumption and inflammatory biomarkers (5-8). In the present study, we also observed that the LG diet led to significant reductions in TC and LDL-C compared to the HA diet. As mentioned in Chapter 2, the significance may be attributed to the higher fiber and lower cholesterol consumptions as well as reduced GI and GL during the LG diet. Both the test diets favorably reduced fasting leptin levels. Both test diets had higher concentrations of vitamin D, which has been shown inversely associated with plasma leptin levels (9).

**Strengths**

As mentioned earlier, the present study has several strengths. The LIFE Study was designed to evaluate the effects of a high legume low glycemic index diet on biomarkers of inflammation and insulin resistance. Therefore, we used the same or similar foods to design our menus (see Appendix E for details). For example, subjects had tuna pasta salad as lunch during the HA diet which was replaced by Italian bean and tuna salad during the LG diet. For dinner, the HA diet provided ginger chicken and rice, 2% milk, broccoli with butter, while the LG diet provided ginger chicken and beans, 2% milk, and broccoli and cauliflower with butter. The ingredients of the two ginger chicken dishes were the same except that the substitution of beans for rice. Therefore, the results that we observed were mostly attributed to differences in legume consumption.

We implemented a randomized, cross-over controlled feeding study design to test our hypotheses. Each participant served as his own control therefore minimized individual variation. Legume consumption was higher than the recommendations (21 vs
14 g/1000 kcal per day) yet the diet was well-tolerated. Both two diets were isocaloric and were matched with similar percentages of energy from total fat, saturated fat, carbohydrate, and protein. Additionally, subjects’ body weight was carefully monitored to rule out the influence of weight loss on the changes in study endpoints.

Limitations

There are some limitations to our study including potentially subjective classification of insulin sensitivity/resistance, lack of direct inference of polyp recurrence, and short compliance breaks between the two dietary periods.

Potentially subjective classification of insulin sensitivity/resistance

The present study defined insulin resistance by HOMA-IR index, which has been widely used for clinical evaluation of insulin resistance; however, the cutoff value used in our study and others is somewhat subjective. In the prospective population-based Bruneck Study (10) of 888 healthy Italian men and women aged 40-79 years, people in the top quintile of HOMA-IR distribution (HOMA-IR ≥ 2.77) were considered insulin resistant. In contrast, in the prospective Turkish Adult Risk Factor Study (11) of 1534 men and women, insulin resistance was defined by the upper quartile of HOMA-IR distribution (HOMA-IR > 2.245). In the cross-sectional Study of Inherited Risk of Coronary Atherosclerosis (SIRCA) (12) including 840 healthy U.S. men aged 30-65 years or women aged 35-70, the cutoff value for insulin resistance was HOMA-IR > 2.114. Recently, two clinical trials designated subjects with a HOMA-IR value higher than 2.6 as insulin resistant (13, 14). The LIFE Study population used the same value as
the cutoff and as a result 28 subjects were designated as insulin resistant. The euglycemic insulin clamp technique is considered the gold standard for evaluating insulin sensitivity/resistance but the technique requires a steady IV infusion and is burdensome for subjects. Though HOMA-IR may be arbitrary, studies show that it is favorably correlated to the estimates based on the euglycemic insulin clamp technique (15, 16).

**Inference for polyp recurrence**

The present study was not designed to evaluate the relationship between a high legume low glycemic index diet and recurrence of colorectal polyps or cancer incidence. The development of colorectal polyps/cancer could take decades, and a 4-week dietary intervention may not be long enough to make a change. For example, both the Polyp Prevention Trial (PPT) (17) and its continued follow-up study (PPT-CFS) (18) failed to show any effects of high-fiber, high-fruit and -vegetable, and low-fat dietary regimen on the recurrence of adenoma after 8-year follow-up.

**Short compliance breaks**

A cross-over design was used for the present study. Cross-over studies compare a given subject’s results on all treatments and assume that changes in variables of interest are due to the dietary intervention rather than subject heterogeneity. Cross-over designs may be subject to both carry-over and period effects. Carry-over effects happen when the effects observed in an intervention period influence the results in the next period. In pharmacological research, the rule of thumb is that the washout period is about five times the length of the half-life of a pharmaceutical product. There is no the similar rule of
thumb in human clinical feeding studies. Our compliance break was set to up to 3 weeks based on previous human feeding studies. We know that four weeks is long enough to change dieting-induced changes in circulating lipids and lipoproteins (19). In this case, even if there might be a carry-over effect from the previous period, we would still have sufficient time to observe changes due to the dietary intervention. Additionally, we analyzed the potential carry-over effects for each variable and we observed no statistically significant carry-over effects. However, we did observe a sequence effect; all subjects had greater changes in biomarkers during the first feeding period than the second feeding period, regardless of diet order. In the future, a longer compliance break might be incorporated to minimize sequence effects.

**Future Directions**

The following considerations may raise the knowledge of legume consumption and the effects on insulin resistance and inflammation.

**Dose-response of high legume consumption**

According to NHANES III (20), daily dietary fiber consumption is about 14-15 g for Americans adults (half the fiber content of the legume enriched diet used in the LIFE study). The LIFE study was not designed to test whether different levels of legume consumption would elicit similar responses on our outcome variables. Future studies should evaluate whether there is a dose-response to high legume consumption.

**Post-meal effect and time course**
The present study was conducted to evaluate the long-term effect of the legume-enriched diet but post-prandial effects were not assessed. At the midpoint of each dietary period, the subjects provided fasting blood samples then consumed either a high GI or low GI breakfast after which 2-hour post-meal blood samples were collected for measurement of study endpoints. However, no legumes were consumed at breakfast; therefore, we were not able to interpret the effect of legume consumption on the changes of the above markers. Additionally, the present study only tracked the concentrations of the biomarkers at fasting and two hours after the breakfast. We know that after food consumption glucose levels increase, peaking approximately an hour after a meal, before declining to normal levels within two hours. It is possible that the high fiber legume-enriched diet may delay macronutrient digestion thus retarding glucose release leading to changes in biomarkers, such as insulin, C-peptide, CRP, and gut hormones within or beyond the two hours. In the future, we should provide beans with breakfast test meal and should track the concentrations of the biomarkers within a time course experiment with multiple blood collections.

**Other gut hormone candidates on satiety**

Obese people are at increased risk for a number of chronic diseases, e.g. CVD, type 2 diabetes, and cancers in breast, colon, and rectum. Legumes are low energy density foods with low glycemic index. Daily increased fiber consumption from legumes may increase satiety, alter food intake and dietary patterns, and shift body metabolism. Therefore, it is important to evaluate the changes in gut hormones and biomarkers of insulin resistance and inflammation.
The present study evaluated the diet effects on the changes in fasting plasma leptin, ghrelin (total), and CCK concentrations under conditions of weight maintenance. We observed significant reductions in plasma, flat values for ghrelin, and no biological changes in CCK (data not shown). Other hormones such as peptide YY (PYY) and glucagon-like peptide (GLP-1) would also be included to evaluate the relationships between these gut hormones and biomarkers of insulin resistance and inflammation.

**Target group and possible study designs**

The present study included male subjects; therefore, the results may not be generalizable to the whole population. In future studies, we might evaluate the effects of the dietary regimen on the same study endpoints within a similar population of women. Other relevant research might be to conduct a community-based study; thus testing the feasibility of providing dietary advice for the consumption of a high legume, fermentable fiber, and low glycemic index diet and the effects on biomarkers of lipid status and other related endpoints among free-living people. Additionally, parallel-arm studies with different levels of legume consumption might be conducted to test for a legume dose-response on the aforementioned endpoints.
References


APPENDIX A

INFORMED CONSENT FORM FOR CLINICAL RESEARCH STUDY

Title of Project: Legume Feeding Study

Principal Investigator: Terry Hartman, Ph.D., R.D.
Department of Nutritional Sciences
5A Henderson
Penn State University
University Park, PA 16802
814-865-8747
Email: tjh9@psu.edu

Principal Investigator: Elaine Lanza, Ph.D.
Laboratory of Cancer Prevention
Center for Cancer Research
National Cancer Institute
National Institutes of Health
Bethesda, MD 20892
301-594-2933
Email: el33t@nih.gov

Co-Investigators
Jan Ulbrecht, M.D.
Penny Kris-Etherton, Ph.D., R.D.
Mihai Covasa, Ph.D.
Carla Miller, Ph.D., R.D.

Study Personnel: Deborah Bagshaw, Clinical Coordinator
814-863-8056
Email: ddm108@psu.edu

Jamie (Zhiying) Zhang, Study Assistant
Email: zzz102@psu.edu

Sarah Mason, Study Assistant
Email: sarah.a.mason@gmail.com

This is to certify that you, ________________________________ (print your name), have been given the following information regarding participation as a volunteer in a program of investigation under the supervision of Dr. Terry Hartman.

This consent form may contain words you do not understand. Please ask the study personnel to explain any words or information you do not clearly understand.
Purpose of the Study

You have been invited to participate in a research study to evaluate the effect of legumes (dried beans) on markers of inflammation, insulin resistance and risk factors for colorectal cancer. This clinical research study, the Legumes Feeding Study, is being sponsored by the National Cancer Institute, National Institutes of Health in collaboration with Penn State University. Large bowel polyps are tumors of the lining of the large bowel, which includes both the colon and rectum. Scientists now believe that most cancers of the large bowel develop from these polyps. Only a small percentage of these polyps actually develop into cancer, and the current medical practice is to remove all large bowel polyps. However, individuals with a history of colorectal polyps have an increased risk of developing another polyp. This study is evaluating two factors known to increase the risk of colorectal polyps and colon cancer. These risks factors are elevated blood insulin and glucose levels and elevated levels of markers of inflammation, such as C-reactive protein. This study will test whether a high legume (dried beans) diet will reduce levels of insulin, glucose and markers of inflammation as well as effects on other measures of health including cholesterol. This new study may provide important information about the health effects of legumes. There is expected to be 68 men enrolled in this study.

Procedures to be followed

If you agree to participate in this study, your participation will last for 11 or 15 weeks total (depending on if you choose to participate in the weight loss diet period). There will be two required 4-week diet periods followed by an optional third 4-week diet period in which you will be allowed to lose weight. You will take an approximate 3-week break between each of the first two diet periods. You will be given all of your food to consume during the three 4-week diet periods. During one of the 4-week periods your will consume a healthy, typical American diet, during the second 4-week period you will consume approximately 1 1/2 cups cooked legumes, such as pinto, baked and navy beans along with a healthy diet. You will be asked to maintain your body weight during these first two study periods. During the third 4-week period, if you chose to participate, you will be given the same 1 1/2 cup cooked legumes, but weight loss will be allowed. You will continue to eat the diet provided that includes the 1 1/2 cup of legumes each day, but you will be instructed to only eat until you feel satisfied. There will be no break before participating in the third diet period.

Screening Procedures

If you wish to participate in this study, you will need to undergo a screening evaluation at the GCRC clinic on the Penn State Campus. During this visit, after giving your consent (in this document) you will have your height, weight, and blood pressure checked by study staff or the nurses at the clinic. You will fill out a questionnaire about your health history and contact information. You will also have a fasting blood sample taken to determine your overall health (including cholesterol levels and lab tests for heart and liver function). Fasting insulin and glucose will also be measured to determine if you are insulin resistant (this means you have a problem with how your body uses glucose and insulin). You will receive the results of the screening procedures whether or not you are admitted into the study. A clinician at the clinic will review all of the results to determine your eligibility to participate in the study. Within about a week of the screening day, you will be informed if you are eligible to participate in the study. If you are eligible and still want to participate, you will be asked to return to the GCRC to have
your energy needs determined (described below), have your waist circumference measured and then told at what later date to report to begin the diet period. During this second visit, you will be instructed about the 3 phone calls you will receive by study staff asking about your daily food intake. These calls will take place over a 7-10 day period. Each call should last about 20 minutes. At this visit, depending on your schedule, the baseline procedures described below (collection of blood samples, instructions on collecting urine and fecal samples) may be done. If it is more convenient for you to return another day for these procedures, the staff can schedule it that way also. If you are not eligible, you will be informed of that fact and your test results will be sent to your personal physician if you give your permission.

**Feeding Study**

To determine your energy needs (how many calories you need to eat) for the feeding study, a technician at the GCRC will measure resting energy expenditure (Resting Metabolic Rate - the energy used just from the regular activity of the heart and other tissues and organs in your body) when you are fasting and in the early morning while you are lying down, and not moving. Your breathing will be measured under a special clear plastic hood, which covers the head. You will be able to communicate with the technician at all times while under this hood. This process takes approximately 90 minutes and the information will be used to plan meals that will maintain your current weight.

During the feeding diet periods, you should eat only those foods and beverages provided (some non-caloric beverages are allowed for free choice). Irrespective of the diet you are on, you will come to the GCRC dining room Monday through Friday for breakfast or dinner, where meals will be prepared and provided. The other meals and snacks will be packed for you to take and eat at a place of convenience. You can request to have all of your food packed each day for consumption at home but you must still report to the diet center to check in and pick up the food. On Friday evenings, you will be given a cooler that contains Friday dinner and Saturday and Sunday meals and snacks. You will be required to appropriately refrigerate and store all foods provided for take-out.

You will be weighed regularly at the GCRC site and should provide the staff dietitian with information about any non-study foods eaten, any study foods not eaten, and caffeine (limited to five, caffeine-containing beverages/day) and alcohol consumption (limited to 2 drinks/week). Other than this, you are supposed to eat all of the food given to you and only the foods given to you and nothing else. If for some reason you fail to do this, it is important to tell the study staff that you did not follow protocol so they can make a note of it in your records. The information provided to the study coordinators will be collected on two separate forms; one to be completed daily and one to be completed weekly. It should only take about 5 minutes to complete these forms each day. Your calorie intake may be adjusted over the course of the study in order to maintain your screening body weight. You understand that during the first two 4-week diet periods you are expected to maintain your current weight. The diet is designed to meet your calorie needs and keep your body weight constant. Calorie intake will be adjusted up or down as necessary to maintain your weight. Also, you should try to keep your exercise level constant throughout the whole study.

If you elect to participate in the weight loss portion of the study (the third, 4-week diet period), weight loss will be allowed during this period. You will be required to eat the 1 ½ c. of legumes each day, but you will eat only as much other food that is provided as you wish, depending on your feeling of fullness.

**Midpoint Testing**

At the mid-point of each of the first two diet periods, you will report to the diet center for a fasting blood draw and then will eat a prescribed breakfast meal. Two hours after the meal, you will have an
additional blood sample taken. In addition, you will fill out after each meal that day, a form rating your sense of fullness and satisfaction with the diet.

**Endpoint Testing**

At the beginning and end of the first two 4-week diet periods we will ask you to collect a stool sample and a 24-hour urine sample. You will be provided with all the materials to collect the urine and stool samples. We will draw a small amount of blood for research at the beginning and end of each of the first two 4-week diet periods and at the end of the optional third diet period. We will measure your blood pressure and waist circumference. We will measure substances in the blood thought to be important for the evaluation of inflammation (we will test for blood markers of inflammation in the body like C-reactive protein which your body produces when there is inflammation) and the control of body weight, blood sugar, and blood lipids (hormones, glucose, growth factors, cholesterol and lipids or blood fat levels). The blood samples may be stored for analysis at a future date. The analysis done on your blood for this study will be restricted to those that influence how the body manages inflammation, how the body’s cholesterol levels are affected, how body weight changes, or how the body uses energy or hormones. Should you withdraw from the study, we will keep your blood for analysis unless you notify us in writing that you do not want this.

The total amount of blood withdrawn will be about **15 tablespoons (~230 cc) over 11 to 15 weeks (depending on participation in optional third diet period)**. Approximately 1.5 tablespoons (~20 cc) will be taken at the screening visit and about 2 tablespoons (~30 cc) will be drawn after an overnight fasting each of four times: baseline, and endpoint of each of first two diet periods. In addition, a fasting sample will be drawn at the end of the optional third diet period (~30 cc) and approximately 2 tablespoon (~30 cc combined) will be drawn 2 hours before and after a meal at the midpoint of each of first two diet periods. This blood withdrawal is well below the NIH guideline for a safe amount of blood withdrawal (450 cc-about 40 tablespoons- per 6 weeks). A small bruise may form at any site where the blood is taken. The risk of infection, small blood clot or fainting is very small. The blood samples will be collected from your arm after a twelve-hour fast (consumption of no food or drinks except water). This will be done at the General Clinical Research Center on PSU campus by trained nursing staff. You should not engage in vigorous physical activity 24 hours prior to having your blood taken. Blood samples will be frozen and analyzed at the end of the study (when all subjects have completed the study). The results of the study will only be available at the end of the entire study (which may take up to 2 years). At the end of the study we will provide participants with a summary of the findings. No personal information will be kept with any sample – only an ID# will be assigned and only the Primary Investigator and the Study Coordinator will have access to the ID# assignments with the study files.

Successful completion of this study depends on the total cooperation of the participants. If during the study, you cannot eat the foods provided and/or eat other foods, you may be asked to leave the study. If you cannot participate in the end-of-diet-period testing, you will be asked to leave the study.

**Time Commitment for the Study**

You will spend approximately the following amounts of time in study activities –

- Screening, visit 1 - 45 min
- Screening, visit 2 - 90 min
Eating at the clinic/filling out forms/picking up food – 30-45 min/ 5 days per week for a total of 225 min/wk for 8-12 weeks – total of 2700 min
Baseline clinic visit for blood draw – 30 min
Midpoint clinic visit for blood draw – 180 min each of 3 times for total of 540 min
End –of-diet period clinic visits – 45 min each of 3 times for total of 135 min
Total time for study is approximately 3454 minutes or about 58 hours

**Discomforts and Risks**

**Feeding Study**

The diets used in this study are nutritionally adequate, whole-food diets. Foods will be prepared according to accepted standards of sanitation and provisions are made to ensure the safety of foods provided for off-site consumption. However, it is possible that incorrect food handling during shipping, storage or preparation, if not detected, could result in food-borne illness. Every effort will be made to safeguard against this possibility. Feeding studies that require on-site eating of meals and strict adherence to the diets provided may interfere with social activities centered on eating such as dining in restaurants. While the menus will provide some variety in the diets, the number of food items will be more limited than that available in an average grocery store. The daily menu will be repeated every six days. The limited variety may become boring over the course of the study.

You may experience temporary, minor gastrointestinal distress (gas, feeling bloated, mild stomach cramps) when increasing the amount of legumes in your diet. This problem is temporary until your body adjusts to the added fiber. You will be allowed to take Gas-X for relief if you feel it is necessary.

You may experience some level of embarrassment or discomfort from being asked to collect urine and stool samples. All the containers and instructions will be given to you to help reduce your concerns.

You may experience some level of discomfort during the measurement of your resting energy rate. During this test, you wear a clear, plastic hood over your head and face for about 30 minutes. For some people, this may cause a feeling of being “closed in” or not being able to “catch their breath”. The procedure will be explained in detail and the technician will always be there during the test.

**Blood Sampling**

The risks involved with taking blood include some local pain and bruising where the blood is taken. Well-trained and experienced phlebotomists will be used to take your blood. Blood sampling can also cause light-headedness and dizziness. If this occurs, having you lie flat with your feet raised will alleviate the symptoms. As with any procedure involving taking blood, infection is possible. All precautions will be taken to avoid infection. There is a rare risk of developing a clot or swelling of the vein and surrounding tissue from the blood draw.

**Benefits to Me**

You will be fed a nutritionally adequate diet for up to three 4-week diet periods for a total of 12 weeks. During this time you will receive all food free of cost. You will have a chance to learn the principles of good nutrition and portion control. If you choose to participate in the third diet period, you may lose weight. You will receive all the results of your blood work from screening for the study (for example: cholesterol and insulin levels). At the end of the study a dietician will explain how the diets are
manipulated and how you can implement these changes into your diet, should you wish to continue eating this type of diet. However, no benefit from participation in this study is guaranteed.

**Potential Benefits to Society**

It is hoped that the information gained from this study will increase our understanding of the effects of eating a legume rich, low sugar, high fiber diet and may help to explain why individuals respond differently to a certain type of diet.

**Statement of Confidentiality**

Your participation in this research is confidential. All records are coded with a unique ID number and no names are used. Records containing names or other identifying information are kept under lock at the Investigator’s research area. Only the investigators and their assistants will have access to your identity and to information that can be associated with your identity. All records associated with your participation in the study will be subject to the usual confidentiality standards applicable to medical records. In the event of publication of this research, no personally identifying information will be disclosed. At the end of the study (after all subjects have completed the study), you will be given your laboratory results without cost, and informed of the study results. The following may review and copy records related to this research: The Office of Human Research Protections in the U.S. Dept. of Health and Human Services; The U.S. Food and Drug Administration (FDA) if applicable; The Penn State University Biomedical Institutional Review Board; The Penn State University Office for Research Protections.

**Right to Ask Questions**

Dr. Hartman or any of the research staff are available to answer any questions that you have at the time of your participation in this study or if you have questions in the future. You will be informed of any new information that may affect your willingness to participate. You may call the Office for Research Protections (814-865-1775) if you need further information about your rights as a research participant.

If the Primary Investigator or study staff becomes aware of new information or research findings that might impact your willingness to participate in this study, you will be given that information. You will be given the opportunity to ask any questions you might have and to decide if you want to continue to participate in the study.

**Compensation**

You will receive all of your food at no cost to you during the diet periods. For your time and participation in the study you will receive monetary compensation of $1000. This will be pro-rated as follows: $200 for completing the eating period and all testing procedures for the first 4 week feeding period, $600 for completing the eating period and all testing through the second 4-week diet period and another $200 for completing the testing for the third diet period. If you are an employee of Penn State University the compensation is treated as taxable income and therefore taxes will be taken from the total compensation amount. If you are not employed by Penn State University, total payments within one calendar year that exceed $600.00 will require the University to annually report these payments to the IRS. This may require you to claim the compensation that is received for participation in this study as taxable income.

**Injury Statement**

Medical care is available in the event of injury resulting from research but that neither financial compensation nor free medical treatment is provided. You are not waiving any rights that you may have
against the University for injury resulting from negligence of the University or the investigators. For further information about this, you may call the Office of Research Protections at 814-865-1775.

**Voluntary Participation**

Participation in this study is voluntary, and you may decline to answer any questions during the screening process or during the study. Refusing to answer a question may keep you from being able to participate in the study. You may withdraw from this study at any time by notifying the investigators or other study personnel. Your withdrawal from this study or refusal to participate will in no way affect your care or access to medical services. You may be asked to leave the study at any time if you do not comply with the study protocol.

In the event that abnormal lab test results are obtained during the study, you will be informed as quickly as possible of these results and instructed to contact your private physician for further assessment. The lab test results will be made available to your private physician at your request.

This is to certify that you are 18 years of age or older and consent to and give permission for your participation as a volunteer in the study entitled “Legume Feeding Study”. You will receive a signed copy after you have read and understand the contents of this consent form.

<table>
<thead>
<tr>
<th>Signature of Volunteer</th>
<th>Date</th>
<th>Printed Name of Volunteer</th>
</tr>
</thead>
</table>

I, the undersigned, have defined and explained the study involved to the above volunteer.

<table>
<thead>
<tr>
<th>Signature of Investigator</th>
<th>Date</th>
</tr>
</thead>
</table>

In addition the main part of the research study, there is an optional part of the research. You can participate in the main part of the research without agreeing to take part in this optional part.

**Storage of Leftover Blood Samples for Future Research Studies**

As part of this study, we are obtaining blood from you. If you agree, the research team would like to store leftover samples of your blood that is collected so that your blood can be studied in the future after this study is over. These future studies may provide additional information that will be helpful in understanding colorectal cancer and other health problems, but it is unlikely that these studies will have a direct benefit to you. Neither your doctor nor you will receive results of these future research tests, nor will the results be put in your health record. Sometimes samples are used for genetic research about diseases that are passed on in families. Even if your samples are used for this kind of research, the results will not be put in your health records. If you have any questions, you should contact Dr. Hartman at 814-865-8747.

Your leftover samples will be labeled with a code number and stored in Dr. Hartman’s locked laboratory or the laboratory of one of the co-investigator’s on this study. If you consent to the collection of samples of
your blood for future research, the period for the use of the samples is unknown. If you agree to allow your blood to be kept for future research, you will be free to change your mind at any time. You should contact Dr. Hartman at 814-865-8747 and let her know you wish to withdraw your permission for your blood to be used for future research. If you do this, any unused blood will be destroyed and not used for future research studies.

You should initial below to indicate your preferences regarding the optional storage of your leftover blood for future research studies.

a. Your samples may be stored and used for future research studies to learn about, prevent, treat or cure colorectal cancer.
   ______ Yes  ______ No

b. Your samples may be stored and used for research about other health problems.
   ______ Yes  ______ No

c. Your samples may be shared with other investigator/groups without any identifying information.
   ______ Yes  ______ No

**Participant:** By signing below, you indicate that you are voluntarily choosing to take part in this optional part of the research.

_________________________  ___________  ___________  ___________
Signature of Participant     Date       Time       Printed Name

**Person Explaining the Research:** Your signature below means that you have explained the optional part of the research to the participant/participant representative and have answered any questions he/she has about the research.

_________________________  ___________  ___________  ___________
Signature of person who explained this optional research   Date       Time       Printed Name
APPENDIX B

INFORMED CONSENT FORM FOR CLINICAL RESEARCH STUDY

Addendum to Primary Consent Form

Title of Project: Legume Feeding Study

Principal Investigator: Terry Hartman, Ph.D., R.D.
Department of Nutritional Sciences
5A Henderson
Penn State University
University Park, PA 16802
814-865-8747
Email: tjh9@psu.edu

Principal Investigator: Elaine Lanza, Ph.D.
Laboratory of Cancer Prevention
Center for Cancer Research
National Cancer Institute
National Institutes of Health
Bethesda, MD 20892
301-594-2933
Email: el33t@nih.gov

Co-Investigators
Jan Ulbrecht, M.D.
Penny Kris-Etherton, Ph.D., R.D.
Mihai Covasa, Ph.D.
Carla Miller, Ph.D., R.D.

Study Personnel:
Deborah Bagshaw, Clinical Coordinator
814-863-8056
Email: ddm108@psu.edu

Jamie (Zhiying) Zhang, Study Assistant
Email: zzz102@psu.edu

Sarah Mason, Study Assistant
Email: sarah.a.mason@gmail.com

This is to certify that you, ________________________________ (print your name), have been given the following information regarding participation as a volunteer in a program of investigation under the supervision of Dr. Terry Hartman. This is an addendum to the primary consent form which you signed at the beginning of your participation in the study.
This consent form may contain words you do not understand. Please ask the study personnel to explain any words or information you do not clearly understand.

PLEASE READ EVERY PAGE CAREFULLY AND INITIAL THE BOTTOM OF EACH PAGE WHEN YOU HAVE HAD ALL OF YOUR QUESTIONS ANSWERED TO YOUR SATISFACTION.

**Purpose of the Study**

This addendum deals with adding a baseline measurement day at the beginning of the optional third diet period. If you would like to take a break of more than 3 days between the second and third diet period, we would like to obtain measurements (weight, blood pressure and obtain a blood sample) to test for any change in your values after you have been eating your normal diet instead of the study diet.

**Procedures to be followed**

If you agree to this additional baseline test day, you will be asked to come to the GCRC clinic on the morning that you will begin the third diet period, after having fasted for 12 hours (no beverage except water and no food), and have a nurse check your weight, blood pressure and draw blood from your arm. Approximately 35 mls (~ 2 tablespoons) of blood will be drawn and tested for the same things as listed for the baseline and end of diet in the original consent form. You will then start the third feeding period.

All other procedures as described in the original consent form will remain the same.

**Time Commitment for the Study**

You will spend approximately the following amounts of time in study activities –

Baseline clinic visit 3 for weight, BP, blood draw – 30 min
Total time for this addition to the study is approximately 30 minutes.

**Discomforts and Risks**

**Blood Sampling**

The risks involved with taking blood include some local pain and bruising where the blood is taken. Well-trained and experienced phlebotomists will be used to take your blood. Blood sampling can also cause light-headedness and dizziness. If this occurs, having you lie flat with your feet raised will alleviate the symptoms. As with any procedure involving taking blood, infection is possible. All precautions will be taken to avoid infection. There is a rare risk of developing a clot or swelling of the vein and surrounding tissue from the blood draw.

**Benefits to Me**

No additional benefit will come to you for participating in this additional blood draw.

**Potential Benefits to Society**

No additional benefit is expected.

**Statement of Confidentiality**

Your participation in this research is confidential. All records are coded with a unique ID number and no names are used. Records containing names or other identifying information are kept under lock at the Investigator’s research area. Only the investigators and their assistants will have access to your identity and to information that can be associated with your identity. All records associated with your participation in the study will be subject to the usual confidentiality standards applicable to medical
In the event of publication of this research, no personally identifying information will be disclosed. At the end of the study (after all subjects have completed the study), you will be given your laboratory results without cost, and informed of the study results. The following may review and copy records related to this research: The Office of Human Research Protections in the U.S. Dept. of Health and Human Services; The U.S. Food and Drug Administration (FDA) if applicable; the Penn State University Biomedical Institutional Review Board; The Penn State University Office for Research Protections.

**Right to Ask Questions**

Dr. Hartman or any of the research staff are available to answer any questions that you have at the time of your participation in this study or if you have questions in the future. You will be informed of any new information that may affect your willingness to participate. You may call the Office for Research Protections (814-865-1775) if you need further information about your rights as a research participant.

If the Primary Investigator or study staff becomes aware of new information or research findings that might impact your willingness to participate in this study, you will be given that information. You will be given the opportunity to ask any questions you might have and to decide if you want to continue to participate in the study.

**Compensation**

No additional compensation is available.

**Injury Statement**

Medical care is available in the event of injury resulting from research but that neither financial compensation nor free medical treatment is provided. You are not waiving any rights that you may have against the University for injury resulting from negligence of the University or the investigators. For further information about this, you may call the Office of Research Protections at 814-865-1775.

**Voluntary Participation**

Participation in this study is voluntary, and you may decline to answer any questions during the screening process or during the study. Refusing to answer a question may keep you from being able to participate in the study. You may withdraw from this study at any time by notifying the investigators or other study personnel. Your withdrawal from this study or refusal to participate will in no way affect your care or access to medical services. You may be asked to leave the study at any time if you do not comply with the study protocol.

This is to certify that you are 18 years of age or older and consent to and give permission for your participation as a volunteer in the study entitled “Legume Feeding Study”. You will receive a signed copy after you have read and understand the contents of this consent form.

_______________________________      ___________  
Signature of Volunteer                  Date                  Printed Name of Volunteer

I, the undersigned, have defined and explained the study involved to the above volunteer.

____________________________________  ____________________
Signature of Investigator                  Date
APPENDIX C

Telephone Interview Form

Date ____________

Interviewer: _______________

Legume Study

Before asking any questions, please read the following paragraph to obtain verbal consent to conduct the telephone interview:

“We received your telephone message that you are interested in participating in the ‘Legume Feeding Study’. I will first read a brief description of the study. In this study we will give you all foods and beverages for 11 or 15 weeks. There will be three, 4-week feeding periods (2 required, one optional) with a 3-week break in-between for a total time commitment of 11-15 weeks. During the 4 week feeding periods we will ask you to eat your weekday breakfast (6:30-9:30 am) or dinner (4:00-6:30 pm), from Monday to Friday, in the dining room of the Research Center on the Penn State campus. Your lunch and other meals and a snack will be packed for take out on weekdays. On the weekends your food will be packed in a large cooler that you will pick up on Friday afternoon. All the foods provided will be typical foods available in local grocery stores. We will ask that you not consume any other foods or beverages other than those provided during each 4-week interval. If needed to accommodate your schedule, food could be packed out for a day or two for you. During one required diet period you will eat on a typical American diet. On the other required diet period, approximately 1 and 1/2 cup of dry beans, such as pinto, navy and kidney beans will be included in your diet. We will draw blood from you 15 times, collect stool samples 4 times, and collect 24 hour urine samples 4 times during the study. The collections will be at the beginning and end of each of the two diet periods, except for a blood draw after two weeks of each diet period. Please note that during the first 2 of the 4-week diet periods you will be weighed regularly to ensure you are not gaining or losing weight. During the final 4-week diet period weight loss will be permitted. You should be aware that the compensation for this study ($1000) is considered income. If you are a Penn State employee it will be taxed, if you are not, it is reportable income.

YES _____ (continue with interview)
NO _____ (thank them for their time and interest)

“I will now ask you a series of questions about your past medical history and your current lifestyle. If you agree to answer these questions, and it is then determined that you meet the criteria for this study, we will schedule you for a screening visit. Are you willing to answer these questions which will take about 15 minutes?”

YES _____ (continue with interview)
NO _____ (thank them for their time and interest)

1. Please give us your:

Name ____________________________________ Date of Birth ____________

Home address ____________________________________________
Daytime Phone# _____________________  Evening Phone # ___________________

2. What is your age? _______
   Your Height (ft and in)___________
   Your Weight (lbs) _____________

   [Interviewer]
   Age between 35 and 75 y  □ Yes  □ No
   BMI 20-37  □ Yes  □ No

3. Have you donated blood in the past 2 months? □ Yes  □ No
   If yes, how long has it been since you donated? ____________
   If not, would you abstain from donating blood during the study? □ Yes  □ No

4. Have you lost any weight during the past 6 months? □ Yes  □ No
   If yes, how much:______________________________

   [Interviewer]
   Wt Loss > 10% body wt  □ Yes  □ No

5. Are you currently on a weight-loss diet or program? □ Yes  □ No
   If yes, please specify: _____________________________
   How long have you been on this weight loss diet? ________
   How much weight have you lost? ________

7. Will you be in the area for the entire 15 weeks of the study? □ Yes  □ No

8. Do you have access to the following appliance at home:
   Refrigerator  □ Yes  □ No
   Freezer  □ Yes  □ No
   Microwave, oven or toaster oven  □ Yes  □ No

9. Do you have any of the following medical conditions:
   a. heart disease  □ Yes  □ No
   b. stroke  □ Yes  □ No
   c. TIA (mini stroke)  □ Yes  □ No
   d. diabetes  □ Yes  □ No
   e. high blood pressure  □ Yes  □ No
   f. renal or kidney disease  □ Yes  □ No
   g. rheumatoid arthritis  □ Yes  □ No
   h. gastrointestinal disease (such as Crohn’s disease, irritable bowel syndrome, ulcer or history of bowel surgery, lactose intolerance).  □ Yes  □ No
1. blood clotting disorder  □ Yes □ No
2. liver disease or cirrhosis  □ Yes □ No
3. any condition that requires the use of steroids  □ Yes □ No
4. gout (requiring treatment)  □ Yes □ No
5. anemia (or sickle cell anemia)  □ Yes □ No
6. lung disease (such as bronchitis, emphysema, asthma)  □ Yes □ No
7. cancer within the last 10 years  □ Yes □ No
8. thyroid disease  □ Yes □ No
9. Problems with immune system (hepatitis, AIDS)  □ Yes □ No
10. any other medical condition not specified in this list  □ Yes □ No
   specify ________________________________

Explain any “yes” answers: ________________________________________________
____________________________________
__________________________________________________________________

10. Do you take any medication prescribed by a doctor? (This includes medications for any diseases, any type of pain medicine, and any drugs for treatment of depression or other mental health problems.)
   □ Yes □ No

   If yes, please specify the type of medication used, duration of use and reason:
   ________________________________________________________________
   ________________________________________________________________
   ________________________________________________________________

11. Do you take any cholesterol-lowering medication? □ Yes □ No
    Example: Zocor, Lovastatin, Zetia, Ezetimibe, Questran, Colestid, Orlistat

    If yes, please specify the type of medication used, duration of use and reason
    ________________________________________________________________
    ________________________________________________________________

12. Are you taking any OTC cholesterol-lowering substances? □ Yes □ No
    Example: psyllium, fish capsules, soy lecithin, phytoestrogen: if so, what? ________________

13. Do you take any medication not prescribed by a doctor? Or any type of nutritional supplement, herb or vitamin? □ Yes □ No

    If yes, please specify the type of medication used, duration of use and reason:
    ________________________________________________________________
    ________________________________________________________________

    If yes, are you willing to discontinue use during the study? □ Yes □ No

14. Do you have any food or nut allergies? □ Yes □ No

    If yes, please specify foods:
    ________________________________________________________________
15. Are you allergic to latex? □ Yes □ No

16. Are you lactose intolerant or allergic to dairy products? □ Yes □ No

17. Do you have any food restrictions related to religious practices? Or are there any foods you refuse to eat? □ Yes □ No
   If yes, please specify:
   ________________________________________________

18. Are you on a special diet prescribed by a doctor or self-prescribed? □ Yes □ No
   If yes, please specify (specifically ask about vegetarian)
   ________________________________________________
   Interviewer Vegetarian □ Yes □ No

19. Do you exercise intensely more than 10 hours a week or play sports regularly? □ Yes □ No
   If yes, please specify: ________________________________

20. Do you currently smoke? □ Yes □ No
    If no, have you ever smoked before? □ Yes □ No
    Explain: _________________________________________

21. Do you consume alcohol? □ Yes □ No
    If yes, how much/how often? __________________________

SUBJECT MAY BE CONSIDERED ELIGIBLE FOR THE STUDY IF NO BOLDED RESPONSES ARE CIRCLED ON THE TELEPHONE INTERVIEW FORM

If subject is eligible, schedule visit to General Clinical Research Center for Clinic Visit 1.
□ Yes, subject eligible Date of screening: __________________________
□ No, subject is not eligible – Reason: __________________________
   __________________________________________________________
Give the subject directions to the General Clinical Research Center in Noll Lab on the PSU campus. Please have them meet the study staff at the 2nd floor nurse’s station. Enter the GCRC through the door that faces Atherton street. Instruct them to put on their flashers, go to the nurse’s station to get a parking permit and then return to their car and put the permit on their mirror and turn off their flashers. The first visit will take approximately 1 hour to complete all of the paperwork and testing.
APPENDIX D

Bean Recipes

**Mexican Salad**

2 cups cooked brown rice  
3 cups cooked black beans  
6 cups chopped lettuce  
1 cup grated cheddar cheese  
2 cups salsa  
4 Tbsp. olive oil

Serve all ingredients individually to assemble as desired.

Nutrients for 4 servings:

<table>
<thead>
<tr>
<th>Energy (kcal)</th>
<th>Fat (g)</th>
<th>CHO (g)</th>
<th>Protein (g)</th>
<th>dfib (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>562</td>
<td>25.00</td>
<td>64.18</td>
<td>23.66</td>
<td>18.31</td>
</tr>
</tbody>
</table>
**Bean and Broccoli salad**

3 cups cooked kidney beans  
2 ½ cups raw broccoli  
6.5 Tbsp. Kraft Zesty Italian dressing

Mix all ingredients and chill. Serve cold.

<table>
<thead>
<tr>
<th>Nutrients for 4 servings:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
</tr>
<tr>
<td>274</td>
</tr>
</tbody>
</table>
**Bean and Veggie Salad**

3 cups cooked kidney beans
2 cups raw tomatoes
1 cup raw sweet green pepper
½ cup raw green onion
8 Tbsp. Kraft Zesty Italian dressing
1 Tsp. ground cumin
4 cups lettuce

Combine all but last ingredient. Chill and serve over lettuce.

**Nutrients for 4 servings:**

<table>
<thead>
<tr>
<th>Energy (kcal)</th>
<th>Fat (g)</th>
<th>CHO (g)</th>
<th>Protein (g)</th>
<th>dfib (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>320</td>
<td>12.23</td>
<td>42.69</td>
<td>13.67</td>
<td>11.84</td>
</tr>
</tbody>
</table>
**Marcia Frifield’s Black Bean Salad**

¼ cup chopped raw onion
1 cup raw sweet green pepper
3 Tbsp. lime juice
3 Tbsp. olive oil
1.5 Tsp. ground cumin
3 Tbsp. fresh cilantro
3 cups cooked black beans

Mix all ingredients and chill. Serve cold.

<table>
<thead>
<tr>
<th>Nutrients for 4 servings:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
</tr>
<tr>
<td>--------------</td>
</tr>
<tr>
<td>275</td>
</tr>
</tbody>
</table>
**Italian Bean and Tuna Salad**

3 cups cooked kidney beans  
4 Tbsp. fresh parsley  
1/3 cup cider vinegar  
1/4 cup olive oil  
2 cups canned tuna, water pack, drained

Combine all ingredients and chill. Serve cold.

**Nutrients for 4 servings:**

<table>
<thead>
<tr>
<th>Energy (kcal)</th>
<th>Fat (g)</th>
<th>CHO (g)</th>
<th>Protein (g)</th>
<th>dfib (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>381</td>
<td>14.82</td>
<td>31.68</td>
<td>31.26</td>
<td>8.62</td>
</tr>
</tbody>
</table>
**Bean and Rice Soup**

1 cup cooked carrots  
4 cups beef broth  
1 Tsp. dried thyme  
5 Tsp. unsalted butter  
3 cups cooked black beans  
1 cup cooked brown rice

Combine first 4 ingredients and heat till carrots are tender. Add in cooked beans and cooked rice. Simmer for 20-30 minutes. Add other seasoning to taste.

<table>
<thead>
<tr>
<th>Nutrients for 4 servings:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
</tr>
<tr>
<td>---------------</td>
</tr>
<tr>
<td>298</td>
</tr>
</tbody>
</table>
Southwest Red Bean Soup

2 Tbsp. olive oil
2/3 cup cooked sweet red peppers
½ cup cooked onion
4 ½ cups low sodium chicken bouillon (broth)
2 Tsp. ground cumin
1/3 cup canned tomatoes
1 Tbsp. unsalted butter
2 cups cooked barley
3 cups cooked kidney beans

Sauté onion and peppers in oil and butter. Add in cumin, diced tomatoes and broth. Simmer for 20-30 minutes. Add in cooked beans and cooked barley and heat for another 10 minutes.

Nutrients for 4 servings:

<table>
<thead>
<tr>
<th>Energy (kcal)</th>
<th>Fat (g)</th>
<th>CHO (g)</th>
<th>Protein (g)</th>
<th>dfib (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>428</td>
<td>12.61</td>
<td>62.39</td>
<td>20.83</td>
<td>14.20</td>
</tr>
</tbody>
</table>
Chicken and Pasta with Beans

4 oz. skinless chicken breast
3 ½ cups cooked whole wheat spaghetti noodles
3 cups cooked whole kidney beans
2 cups cooked broccoli
2 Tbsp. chopped garlic
2 Tbsp. unsalted butter
8 Tsp. olive oil
4 Tsp. grated parmesan cheese

Cut chicken into bite-sized pieces. Sauté in oil and butter till chicken is no longer pink. Add in frozen broccoli and garlic and simmer till the broccoli is just thawed and bright green. Add in cooked beans and serve over cooked pasta. Sprinkle with Parmesan.

Nutrients for 4 servings:

<table>
<thead>
<tr>
<th>Energy (kcal)</th>
<th>Fat (g)</th>
<th>CHO (g)</th>
<th>Protein (g)</th>
<th>dfib (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>515</td>
<td>17.66</td>
<td>66.87</td>
<td>26.90</td>
<td>13.47</td>
</tr>
</tbody>
</table>
**Chili with Beans**

3 Tbsp. olive oil  
4 cups tomato sauce  
1/3 packet dry mix taco seasoning packet  
1 cup canned tomatoes  
¾ cup cooked celery  
1/3 cup cooked sweet green pepper  
3 cups cooked kidney beans  
1 2/3 cups cooked barley

Sauté celery and green pepper in oil until tender. Add in tomato sauce, taco seasoning and diced tomatoes. Simmer for 20-30 minutes. Add cooked beans and cooked barley and heat for 10 more minutes.

---

**Nutrients for 4 servings:**

<table>
<thead>
<tr>
<th>Energy (kcal)</th>
<th>Fat (g)</th>
<th>CHO (g)</th>
<th>Protein (g)</th>
<th>dfib (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>446</td>
<td>11.89</td>
<td>72.40</td>
<td>18.13</td>
<td>17.16</td>
</tr>
</tbody>
</table>
**Easy Baked Beans**

3 cups cooked pinto beans
2/3 cup raw onion
1 cup low sodium ketchup
½ Tsp. ground ginger
4 Tbsp. unsalted butter

Preheat over to 350° F. Combine all ingredients. Cover with foil and bake in Pam-sprayed baking pan for 30-45 minutes.

Nutrients for 4 servings:

<table>
<thead>
<tr>
<th>Energy (kcal)</th>
<th>Fat (g)</th>
<th>CHO (g)</th>
<th>Protein (g)</th>
<th>dfib (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>288</td>
<td>5.13</td>
<td>51.18</td>
<td>12.92</td>
<td>11.86</td>
</tr>
</tbody>
</table>
**Chicken and Bean Cassoulet**

5 oz. raw skinless chicken breast
3 cups cooked navy beans
1 Tsp. fresh garlic
1 Tbsp. olive oil
2/3 cup cooked onion
½ cup low sodium chicken bouillon (broth)
1 ½ Tsp. dried thyme
2 cups canned tomatoes
¼ cup plain bread crumbs
4 Tbsp. dry parmesan cheese
2 Tbsp. unsalted butter

This has been a favorite of everyone! Preheat oven to 375° F. Spray over-proof casserole dish with Pam and assemble ingredients in this order: bite-sized chicken pieces, cooked beans, garlic, onion, thyme, oil, broth and tomato. Sprinkle with Parmesan and breadcrumbs and dot with butter. Cover with foil and bake for 45 minutes or until internal temperature of 165° F. is reached.

<table>
<thead>
<tr>
<th>Nutrients for 4 servings:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
</tr>
<tr>
<td>413</td>
</tr>
</tbody>
</table>
**Ginger Chicken and Beans**

4 oz raw skinless chicken breast  
¼ cup olive oil  
½ cup cooked onion  
2/3 cup cooked sweet green pepper  
1/3 Tsp. ground ginger  
1 Tsp. fresh garlic  
2 Tsp. white all-purpose flour  
½ Tsp ground allspice  
1/3 Tsp. ground cumin  
1/8 Tsp. nutmeg  
1/8 Tsp ground black pepper  
1 ¼ cups low sodium chicken bouillon (broth)  
3 cups cooked kidney beans

Cut chicken into bite-sized pieces and sate in oil until all pink is gone. Mix in dry ingredients, onion, green pepper, garlic and broth. Simmer for 20-30 minutes. Add in cooked beans and heat for another 10 minutes.

<table>
<thead>
<tr>
<th>Nutrients for 4 servings:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>Fat (g)</td>
</tr>
<tr>
<td>----------</td>
<td>---------</td>
</tr>
<tr>
<td>361</td>
<td>15.74</td>
</tr>
</tbody>
</table>
Pork Stir-Fry with Beans

3 cups cooked brown rice
1 cup cooked kidney beans
2/3 cup cooked onion
1.5 cups canned-drained mushrooms
3 cups cooked fresh broccoli
6 Tsp. sesame oil
12 oz. raw roast pork (loin)
3.5 Tbsp. low sodium soy sauce
6 Tsp. unsalted butter

Precook pork by baking or stir frying first and cut into bite-sized pieces. Stir fry onion, mushrooms, broccoli, and pork in oil and butter until broccoli is crisp-tender. Add soy sauce and serve over steaming hot rice and beans.

Nutrients for 4 servings:

<table>
<thead>
<tr>
<th>Energy (kcal)</th>
<th>Fat (g)</th>
<th>CHO (g)</th>
<th>Protein (g)</th>
<th>dfib (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2184</td>
<td>86</td>
<td>232.8</td>
<td>131.2</td>
<td>54</td>
</tr>
</tbody>
</table>
## APPENDIX E

### Healthy American Diet

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breakfast</strong></td>
<td><strong>Lunch</strong></td>
<td><strong>Dinner</strong></td>
<td><strong>Snacks</strong></td>
<td><strong>Breakfast</strong></td>
<td><strong>Lunch</strong></td>
</tr>
<tr>
<td><em>Chilled OJ</em></td>
<td><em>Tuna Sandwich on Whole Wheat</em></td>
<td><em>Chili</em></td>
<td><em>Bread w/Peanut Butter</em></td>
<td><em>Chilled OJ</em></td>
<td><em>Ham Sandwich</em></td>
</tr>
<tr>
<td><em>Waffles w/Margarine and Syrup</em></td>
<td><em>Baked French Fries w/Ketchup</em></td>
<td><em>Canned Apricots</em></td>
<td><em>Toasted Bagel w/Cream Cheese</em></td>
<td><em>Cheiled Cranberry Juice</em></td>
<td><em>Canned Peaches</em></td>
</tr>
<tr>
<td><em>Turkey Sausage</em></td>
<td><em>Crisp Romaine Salad</em></td>
<td><em>Nilla Wafers</em></td>
<td><em>Cheddar cheese</em></td>
<td><em>Milk</em></td>
<td><em>Milk</em></td>
</tr>
<tr>
<td><em>Chilled Cranberry Juice</em></td>
<td><em>Tuna Pasta Salad</em></td>
<td><em>Ginger Chicken &amp; Rice</em></td>
<td><em>Swiss cheese &amp; crackers</em></td>
<td><em>Baked Flounder w/Tartar Sauce</em></td>
<td><em>Parmesan Rice</em></td>
</tr>
<tr>
<td><em>Crispix Cereal w/Milk</em></td>
<td><em>Canned peaches</em></td>
<td><em>Milk</em></td>
<td><em>Raisin &amp; Date Trail Mix</em></td>
<td><em>Corn</em></td>
<td><em>Canned Peaches</em></td>
</tr>
<tr>
<td><em>Fresh Banana</em></td>
<td><em>Hearty Chicken Chili</em></td>
<td><em>Chicken &amp; gravy</em></td>
<td><em>Mixed Greens Salad</em></td>
<td><em>Whole Wheat Rice</em></td>
<td><em>Milk</em></td>
</tr>
<tr>
<td><em>Toast w/Peanut Butter</em></td>
<td><em>Crisp Romaine Salad</em></td>
<td><em>Stuffing</em></td>
<td><em>Garlic Chicken &amp; Pasta w/Broccoli</em></td>
<td><em>Corn</em></td>
<td><em>Canned Peaches</em></td>
</tr>
<tr>
<td><em>Blueberry muffin</em></td>
<td><em>Chilled Cranberry Juice</em></td>
<td><em>Rice w/ Almonds</em></td>
<td><em>Mixed Greens Salad</em></td>
<td><em>Whole Wheat Bread</em></td>
<td><em>Milk</em></td>
</tr>
<tr>
<td><em>Scrambled eggs</em></td>
<td><em>Chilled OJ</em></td>
<td><em>Broccoli</em></td>
<td><em>Baked Flounder w/Tartar Sauce</em></td>
<td><em>Whole Wheat Bread</em></td>
<td><em>Milk</em></td>
</tr>
</tbody>
</table>

*The menu for the mid-point blood draw day will differ from above*
### Legume Diet

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breakfast</strong></td>
<td><strong>Breakfast</strong></td>
<td><strong>Breakfast</strong></td>
<td><strong>Breakfast</strong></td>
<td><strong>Breakfast</strong></td>
<td><strong>Breakfast</strong></td>
</tr>
<tr>
<td><em>Chilled OJ</em> <em>Scrambled Eggs</em> <em>Bran Chex Cereal w/Milk</em></td>
<td><em>Chilled Apple Juice</em> <em>Bran Chex Cereal w/Milk</em> <em>Oat Bran Bread w/Butter</em></td>
<td><em>Chilled Apple Juice</em> <em>Yogurt</em> <em>Sourdough Toast w/Butter</em> <em>Spinach Omelet</em></td>
<td><em>Raisin Bran w/Milk</em> <em>Fresh Banana</em> <em>Scrambled Eggs</em></td>
<td><em>Bran Chex w/Milk</em> <em>MultiGrain Toast w/Butter &amp; PB</em></td>
<td><em>Omelet</em> <em>Oat Bran Toast w/Peanut Butter</em></td>
</tr>
<tr>
<td><strong>Lunch</strong></td>
<td><strong>Lunch</strong></td>
<td><strong>Lunch</strong></td>
<td><strong>Lunch</strong></td>
<td><strong>Lunch</strong></td>
<td><strong>Lunch</strong></td>
</tr>
<tr>
<td><em>Tuna Sandwich on MultiGrain</em> <em>Bean &amp; Broccoli Salad</em></td>
<td><em>Italian Bean &amp; Tuna Salad</em> <em>Canned Pears</em> <em>MultiGrain Bread w/Butter</em></td>
<td><em>Southwest Bean Soup</em> <em>Salad</em></td>
<td><em>Bean &amp; Rice Soup</em> <em>Crunchy Veggies w/Ranch Dip</em></td>
<td><em>Bean &amp; Veggie Salad</em> <em>Milk</em> <em>Fruit cup</em></td>
<td><em>Hearty Black Bean Soup</em> <em>Cherries</em> <em>Milk</em> <em>Vanilla Pudding</em></td>
</tr>
<tr>
<td><strong>Dinner</strong></td>
<td><strong>Dinner</strong></td>
<td><strong>Dinner</strong></td>
<td><strong>Dinner</strong></td>
<td><strong>Dinner</strong></td>
<td><strong>Dinner</strong></td>
</tr>
<tr>
<td><em>Chili</em> <em>Fresh Spinach Salad</em> <em>Chocolate Cake</em> <em>Milk</em></td>
<td><em>Ginger Chicken &amp; Beans</em> <em>Broccoli &amp; Cauliflower</em> <em>Milk</em></td>
<td><em>Chicken &amp; Bean Cassoulet</em> <em>Vanilla Cake</em> <em>Milk</em></td>
<td><em>Garlic Chicken &amp; Pasta</em> <em>Salad</em> <em>Milk</em></td>
<td><em>Baked Flounder w/Tartar Sauce</em> <em>Whole Wheat Pasta</em> <em>Baked Beans</em> <em>Chocolate Pudding</em></td>
<td><em>Pork Stir-Fry w/Brown Rice</em> <em>Three Bean Salad</em> <em>Milk</em> <em>Oat Bran Bread</em></td>
</tr>
<tr>
<td><strong>Snack</strong></td>
<td><strong>Snack</strong></td>
<td><strong>Snack</strong></td>
<td><strong>Snack</strong></td>
<td><strong>Snack</strong></td>
<td><strong>Snack</strong></td>
</tr>
<tr>
<td><em>Grapes</em></td>
<td><em>Cheddar Cheese</em></td>
<td><em>Fresh Apple</em> <em>Roasted Almonds</em></td>
<td><em>Yogurt w/Strawberries</em> <em>Peanut M&amp;Ms</em></td>
<td><em>Cheddar Cheese</em> <em>Fresh Apple</em></td>
<td><em>Apple</em></td>
</tr>
</tbody>
</table>

*The menu for the mid-point blood draw day will differ from above*

**The first three days you are on the Legume Diet will be slightly different than above*
Figure 1. Plots of serum TC among subjects who ate the legume diet followed by the healthy American diet. Unit: mg/dL.
Figure 2. Plots of serum TC among subjects who ate the healthy American diet followed by the legume diet. Unit: mg/dL.
Figure 3. Plots of serum HDL-C among subjects who ate the legume diet followed by the healthy American diet. Unit: mg/dL.
Figure 4. Plots of serum HDL-C among subjects who ate the healthy American diet followed by the legume diet. Unit: mg/dL.
Figure 5. Plots of serum LDL-C among subjects who ate the legume diet followed by the healthy American diet. Unit: mg/dL.
Figure 6. Plots of serum LDL-C among subjects who ate the healthy American diet followed by the legume diet. Unit: mg/dL.
Figure 7. Plots of serum TG among subjects who ate the legume diet followed by the healthy American diet. Unit: mg/dL.
Figure 8. Plots of serum TG among subjects who ate the healthy American diet followed by the legume diet. Unit: mg/dL.
Figure 9. Plots of plasma leptin among subjects who ate the legume diet followed by the healthy American diet. Unit: ng/mL.
Figure 10. Plots of plasma leptin among subjects who ate the healthy American diet followed by the legume diet. Unit: ng/mL.
Figure 11. Plots of plasma ghrelin (total) among subjects who ate the legume diet followed by the healthy American diet. Unit: pg/mL.
Figure 12. Plots of plasma ghrelin (total) among subjects who ate the healthy American diet followed by the legume diet. Unit: pg/mL.
VITA
Zhiying Zhang

EDUCATION
2003-2005 M.S. Veterinary Sciences in Reproductive Biology, University of Prince Edward Island, Charlottetown, PE, Canada
1997-2001 B.S. Veterinary Medicine, Huazhong Agricultural University, Wuhan, Hubei, China

RESEARCH EXPERIENCE
2005-2010 Graduate Research Assistant, The Pennsylvania State University
2003-2005 Graduate Research Assistant, University of Prince Edward Island, Charlottetown, PE, Canada
2001-2002 Research Assistant, The State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China

HONORS AND AWARDS
2009-2010 Woot-Tsuen Wu Lueng Scholarship in Nutrition
2006-2007 Woot-Tsuen Wu Lueng Scholarship in Nutrition
2005-2006 The Pennsylvania State University Fellowship

ORAL PRESENTATIONS

POSTER PRESENTATIONS/MEETING ABSTRACTS

PUBLICATIONS