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Consumption of a Legume-Enriched, Low-Glycemic Index Diet Is Associated with Biomarkers of Insulin Resistance and Inflammation among Men at Risk for Colorectal Cancer[1,2]

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Abstract

The Legume Inflammation Feeding Experiment is, to our knowledge, the first randomized crossover feeding trial testing the effects of a legume-enriched, low-glycemic index (GI) diet among men characterized for colorectal adenomas and insulin resistance (IR) status. This study was designed to test the effects of a legume-enriched diet compared with a healthy American (HA) diet under weight-stable conditions. The primary objective was to assess effects on C-reactive protein (CRP) and C-peptide levels. The secondary objective was to assess changes by IR status or history of adenomas. A total of 64 men who completed a colonoscopy within the previous 2 y consumed 2 diets in random order each for 4 wk separated by a washout period. The diets were a legume-enriched (250 g/d), low-GI (GI 38) diet and a high-GI (GI 69) HA diet. We measured fasting glucose, insulin, C-peptide, CRP, and soluble tumor necrosis factor-α receptors I and II (sTNFRI/II) at the beginning and end of the diet periods. Participants who consumed both the legume and HA diets had favorably improved CRP (−20.2 and −18.3%) and sTNFRI (−3.7 and −4.4%) concentrations, respectively. The sTNFRII concentrations declined marginally during the legume diet period (−3.8%; $P = 0.060$) and significantly during the HA diet period (−5.1%; $P < 0.001$). Fasting glucose increased significantly during both the legume (+1.8%) and HA (−2.2%) diet periods. Only the changes in glucose differed between the diet periods. Serum C-peptide and plasma insulin levels did not change in participants consuming either diet. Healthful dietary changes can improve biomarkers of IR and inflammation. J. Nutr. 140: 60–67, 2010.

Introduction

Colorectal cancer is the 3rd leading cause of cancer death among both genders in the US (1). The epidemiology of colorectal adenomas closely resembles that of colorectal cancer itself; thus, prevention of adenomas most likely prevents colorectal cancer.

The Polyp Prevention Trial (PPT)[8] was a 4-y multi-center, randomized trial of 1905 participants who had a colorectal adenoma removed prior to randomization (2–4). In a post-trial analysis, legume consumption was significantly associated with adenoma recurrence (5). Those with the greatest increase in dried bean intake had a significantly reduced odds ratio for advanced adenoma recurrence [odds ratio = 0.35 (95% CI, 0.18–0.69); P-trend = 0.001] compared with those with the lowest intakes. Legumes are a rich source of fermentable dietary fibers that are precursors of luminal butyrate with well-known antiinflammatory and antineoplastic properties. Legumes have a low glycemic index (GI) due to their high fiber and high resistant starch content. The GI describes the rate of glucose disposal in blood and is used to compare glycemic responses of different foods. In contrast, glycemic load (GL) considers both the quality and the quantity of carbohydrates consumed (GI × g carbohydrate/serving) (6).

The World Cancer Research Fund/American Institute for Cancer Research's recently released "Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective" recognized obesity, body fatness, abdominal fatness, and physical

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[8] Abbreviations used: CRP, C-reactive protein; GI, glycemic index; GL, glycemic load; HA, healthy American; IR, insulin resistance; IS, insulin sensitive; PPT, Polyp Prevention Trial; sTNFRI/II, soluble tumor necrosis factor-α receptors I and II.
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inactivity as important modifiable risk factors for colorectal cancer (7). This report highlighted evidence linking these exposures to insulin resistance (IR) and type 2 diabetes, which are also positively associated with colorectal cancer (7,8). C-peptide, a marker of insulin production, is elevated in IR and has been associated with colorectal cancer risk (9,10). Tran et al. (11) demonstrated a positive and dose-dependent association between acutely elevated circulating insulin concentrations and increased colorectal epithelial cell proliferation in an animal model. Obesity, IR, and colorectal cancer are increasingly recognized as chronic, low-level, inflammatory states. C-reactive protein (CRP), an acute phase protein and a sensitive marker of subclinical inflammation, has been associated with obesity (12), IR (13,14), diabetes (15), and colon cancer risk (10); however, results for colon cancer are not consistent across studies (16). Recent evidence suggests that IR and inflammation may act synergistically to promote colorectal cancer. An important question is whether lifestyle interventions that can favorably alter IR and inflammation will lower risk for colorectal cancer and, further, whether these effects can be achieved independent of weight loss.

Dietary intake can influence biomarkers of insulin sensitivity and inflammation. Evidence suggests a positive relationship among high-GI or -GL diets with biomarkers of hyperinsulinemia, type 2 diabetes (17–19), and colorectal cancer, particularly among men (20–23). In addition, at least 2 recent studies reported that GL or GI was positively associated with plasma CRP levels among healthy older women (24). In a subset of 224 healthy participants enrolled in the Women's Health Study, Liu et al. (24) observed median plasma CRP concentrations increased across increasing quintiles of dietary GL (CRP = 1.9 mg/L for the lowest and 3.7 mg/L for the highest quintiles of dietary GL, respectively; P-trend < 0.01). Similarly, Levitan et al. (25) reported that participants in a sample of 18,137 women within the highest quintile of GI (median GI 57) had higher CRP concentrations compared with those in the lowest GL quintile (1.9 vs. 1.7 mg/L; $P < 0.001$).

We designed the Legume Inflammation Feeding Experiment to compare the effects of a legume-enriched, low-GI diet to a healthy American diet (HA) on biomarkers related to IR and inflammation in a population of men with known insulin sensitivity and a history of colorectal adenoma status. We hypothesized that the high-legume diet would reduce the rate of carbohydrate absorption, lowering the postprandial glycemic and insulinemic responses, leading to decreased C-peptide and other markers of insulin production.

Participants and Methods

Participants and study design

The Legume Inflammation Feeding Experiment Study was conducted between 2006 and 2008 at the General Clinical Research Center, The Pennsylvania State University. Male participants who had undergone a screening colonoscopy within the past 2 y were recruited with the assistance of local gastroenterologists to represent 4 combinations of adenoma history and IR at baseline: previous history of adenomas with and without IR, and no previous history of adenomas with and without IR. The primary objective was to assess an overall effect of the high-legume, low-GI diet on the 2 primary endpoints, CRP and C-peptide in this population. IR was defined by the Homeostasis Assessment Model [= fasting insulin (μU/mL) $\times$ fasting glucose (mmol/L)/22.5]. Values $>$ 2.61 were considered insulin resistant (26). In addition to having undergone a recent colonoscopy, eligible men had to be 35–75 y of age, nonsmokers, in good health, and without a history of colorectal cancer or bowel resection, the polyposis syndrome, inflammatory bowel disease, or other related bowel conditions. Men also could not be regularly taking prescription or nonprescription preparations that might alter blood concentrations of inflammation markers, insulin, glucose, or lipids and had to be willing to adhere to the study diet. Participants were also asked to keep their activity level constant during the study. All participants signed an informed consent before entering the study. Sixty-six men were enrolled in the study; 2 dropped out within the first week of participation, leaving 64 for analysis. Institutional review boards at the National Cancer Institute, Bethesda, MD and the Pennsylvania State University, University Park, PA approved the study.

Study diets. Participants completed a resting energy expenditure test and 3 d of dietary records to estimate their total energy needs for the study before beginning the 2 test diets. Participants then consumed 2 isocalorically controlled diets in random order, a control (HA) and a high-legume, low-GI diet (legume), each during 2 4-wk periods separated by a washout (2 wk on average). Weight was monitored on each weekday and energy intake was adjusted in 837.4-kJ (200 kcal)[9] increments (diets were designed and menus available for 2000, 2200, 2400, 2600, etc. kcal) as needed to maintain body weight throughout the study. Participants were allowed up to 2 alcoholic beverages/wk while consuming the study diets. Seven-day cycle menus were created for each diet and evaluated for nutrient content using both Food Processor (2005) and the Nutrition Data System for Research (2006, Minneapolis, MN) (27). When the study diets were designed, data for GI and GL were only available in Food Processor; thus, those data were used for calculations, using glucose as the reference. Both test diets provided ~34% of energy from fat (11–12% saturated fat), 18% of energy from protein, and 50% of energy from carbohydrate. The percentage of energy contributed by each of the energy nutrients is calculated using general Atwater factors of 9, 4, and 4 kcal/g for fat, carbohydrate, and protein, respectively, which resulted in the total percent calories not adding to 100% [described in the NDSR Manual (27)]. The high-legume treatment contained ~250 g legumes/d (1 1/2 cups) that were members of the *Phaseolus vulgaris* species, including navy, pinto, kidney, and black beans. These were chosen to minimize nutrient and phytochemical variability in the menu cycle. The legumes provided much of the protein for this diet; the control diet provided more protein as chicken. The control diet included 9 g fiber/1000 kcal and the legume diet 21 g/kcal and cholesterol content of the 2 diets was 98 mg/1000 kcal (control) and 70 mg/1000 kcal. The GI and GL were 69 and 152 for the control diet and 38 and 84 for the legume diet, respectively. All foods and beverages were prepared at the Penn State University General Clinical Research Center (University Park, PA). **Table 1** provides the nutrient composition of each diet. On weekdays, participants consumed 1 meal at the center and other meals and snacks were prepared and packaged for carry-out. All weekend foods and beverages were packaged for consumption at home with written instructions. Diets provided at least 100% of the recommended dietary allowances for vitamins and minerals and supplements were not allowed. Test diet adherence was very good, as indicated by daily compliance questionnaires and only a negligible return of uneaten foods throughout the study.

Laboratory methods. At the beginning and end of each treatment period, blood was collected in the early morning after an overnight fast. In addition, we collected both fasting and 2-h postprandial blood samples at 2 wk (midpoint) of each dietary treatment after the consumption of a typical breakfast to assess the acute effects of the 2 dietary treatments on postprandial biomarkers of interest. Serum and plasma samples were taken and stored at -80°C until the end of the study for analysis. Serum C-peptide and plasma insulin, glucose, high sensitivity CRP, and soluble tumor necrosis factor-α receptors I and II (sTNFRI/II) were measured at the Hershey Medical Center in the laboratory of Dr. Laurence Demers. Insulin was measured by RIA using ^{125}I-labeled human insulin and human insulin antiserum (Linco Research). Glucose was measured via an immobilized enzyme biosensor using the YSI 2300 STAT Plus Glucose and Lactate analyzer (Yellow Springs Instruments). Serum C-peptide was measured by RIA using

[9] 1 kcal = 4.187 kJ.

TABLE 1 Nutrient and food profiles of the experimental diets (means of 7-d menus) compared with prestudy diets among men at risk for colorectal cancer[1]

Nutrient/food group	Prestudy diet	HA (High-GI) diet	Legume (Low-GI) diet	*P*-value
GI[2,4]	60 ± 6[a]	69 ± 3[b]	38 ± 2[c]	<0.0001
GL[2]	165 ± 77[a]	152 ± 8[a]	84 ± 4[b]	0.005
Total fat,[3] *% energy*	35 ± 7	34 ± 1	34 ± 1	0.849
SFA,[3] *% energy*	12 ± 3	11 ± 1	12 ± 1	0.705
Protein,[3] *% energy*	16 ± 3	18 ± 1	18 ± 1	0.053
Carbohydrate,[3] *% energy*	48 ± 8	50 ± 2	51 ± 1	0.604
Total fiber,[2,3] *g/1000 kcal*	9 ± 3[a]	9 ± 1[a]	21 ± 1[b]	<0.001
Cholesterol,[2,3] *mg/1000 kcal*	123 ± 52[a]	98 ± 9[a,b]	70 ± 11[b]	0.010
Total vitamin A activity,[2,3] *retinol activity equivalent, μg/1000 kcal*	353.7 ± 160.0	411.2 ± 81.4	381.3 ± 95.7	0.310
Total carotenoids,[2,3] *μg/1000 kcal*	4756 ± 3147[a]	8272 ± 7465[a,b]	8799 ± 6996[b]	0.031
Vitamin D,[2,3] *calciferol, μg/1000 kcal*	2.3 ± 1.5[a]	3.0 ± 0.9[a,b]	3.5 ± 1.2[b]	0.035
Vitamin E,[2,3] *total α-tocopherol equivalent, mg/1000 kcal*	5.6 ± 3.2	6.3 ± 1.2	6.4 ± 1.8	0.226
Vitamin C,[2,3] *mg/1000 kcal*	39.7 ± 24.3[a]	68.6 ± 21.8[b]	61.8 ± 25.7[b]	0.004
Dietary folate equivalent,[2,3] *μg/1000 kcal*	334.9 ± 154.2	363.4 ± 121.9	444.1 ± 76.2	0.069
Fruit,[2,3] *serving/1000 kcal*	0.7 ± 0.6[a]	0.9 ± 0.4[a,b]	1.3 ± 0.3[b]	0.021
Vegetable,[3] *serving/1000 kcal*	1.4 ± 0.7[a]	1.8 ± 0.5[a,b]	2.2 ± 0.6[b]	0.007
Legume,[2,3] *serving/1000 kcal*	0.1 ± 0.2[a]	0.0 ± 0.0[a]	1.5 ± 0.0[b]	<0.001

[1] Values are the mean + SD, $n = 64$ participants (prestudy) or 7 diet menus (all participants received the same HA and legume 7-d diet menu cycles). Means in a row with superscripts without a common letter differ, $P < 0.05$.
[2] Data were log-transformed for analysis.
[3] 1 kcal = 4.187 kJ.
[4] GI values based on glucose as a reference and are calculated for the mean of daily menus.

reagents provided by Linco Research. High sensitivity CRP and sTNFRI/II were analyzed via sandwich enzyme immunoassay using reagents from ALPCO diagnostics and R&D Systems, respectively. Samples for individual participants were grouped in random order together within the same batch for analysis. The CV estimated from quality control samples across batches were: insulin, 3.9%; glucose, 5.0%; C-peptide, 4.7; CRP, 5.8%; sTNFRI, 6.1%; and sTNFRII, 4.1%.

Statistical analysis. The sample size for the study was based on accruing a total of 60 men who would complete the study (15 in each of the 4 groups). There were 2 major statistical endpoints (primary objectives) in this study: mean change between fasting blood levels of CRP and C-peptide measured at the end of the 4-wk high-legume diet and at the end of a 4-wk HA diet, and difference between the CRP and C-peptide concentrations at the end of each diet period. Jenkins et al. (28) found a mean baseline C-reactive protein value of 2.39 mg/L and observed a (mean + SD) difference of −1.25 mg/L (SE reported as 0.62 and calculated SD is 2.48) among 16 male and female participants after 4 wk on their Portfolio diet. Based on these data, with a total of 60 middle-aged male participants (many expected to have elevated CRP), we estimated 80% power to detect a 42% reduction in the mean CRP measure (from 2.39 mg/L to 1.39 mg/L) from the HA diet to the high-legume diet using a 2-sided paired *t* test conducted at the 0.025 significance level. Based on a small set of data presented in another Jenkins et al. (29) study, we should have high power to detect smaller differences in the serum C-peptide biomarker. The baseline serum C-peptide measurement, presented in Table 3 of their paper, was 0.46 nmol/L (29). A conservative estimate of the SD derived from data in their paper is 0.15. Using this data, we would have 80% power to detect a 13% reduction in the mean serum C-peptide (from 0.46 to 0.40 nmol/L) from the HA diet to the high-legume diet using a 2-sided paired *t* test conducted at the 0.025 significance level.

The experimental diets were compared with the prestudy diet and to each other using 1-way ANOVA to compare the mean of each 7-d menu (legume, HA) to the means for 3-d diet recalls reflecting the participants' prestudy diets. Pairwise comparisons were made with the Tukey test. Baseline characteristics of study participants were compared across groups using 1-way ANOVA. We used linear mixed models to test for differences in biomarker concentrations across treatment groups for our final results. However, analyses were also completed using paired *t* tests with similar results. Participant (subject) was treated as a random effect (i.e. a single random intercept) and diet treatment as a fixed effect designated by 2 indicator variables. These models allow for flexibility, testing for treatment effects as well as examining for period (time) and order effects (i.e. testing for carryover effects) and adjusting for important baseline and time-dependent (i.e. BMI) covariates. Biomarker concentrations were analyzed as untransformed for glucose and the receptors and transformed to the $\log_e$ for C-peptide, insulin, and CRP. We obtained similar results for transformed and untransformed variable concentrations; therefore, we report the results for the untransformed data. We evaluated the effects of other variables, including age, BMI (weight in kg/height in m^2), biomarker status at baseline, treatment period, and treatment order for contribution to overall fit or improvement in the precision of the model. In our initial analyses, Akaike Information Criteria was used to decide whether we should specify compound symmetry or unstructured for our correlation structure. We used unstructured based on these comparisons and because we also thought that the covariance between treatments might be different. We relied upon the likelihood ratio chi-squared test to test for treatment effect. SE of diet treatment estimates from simple models and models that included characteristics potentially associated with the biomarker concentration were compared with evaluate the effect of adjustment on precision. Other covariates such as age, BMI, baseline biomarker status, period, and treatment order did not affect our inferences for diet treatment. Effect modification by treatment order, BMI, and age was assessed by likelihood ratio tests of improvement in model fit after addition of the interaction (cross product) terms to models that included the main effects for diet and the characteristic of interest. Our results indicated that IR status may be an important consideration for the interpretation of the analysis for some biomarkers; therefore, we also conducted analyses stratified by IR status. There was no evidence of this for adenoma status. All analyses were performed using SAS (SAS/Stat version 9.2, SAS Institute). The final results are reported as least-squares means ± SEM. Significance was set at $P \leq 0.05$.

Results

The legume low-GI diet had lower GI and GL and higher fiber concentrations than the other diets (Table 1). Compared with the prestudy diet, both test diets had significantly higher concentrations of vitamin C. The dietary cholesterol concentration was lower for the legume diet compared with the prestudy diet. In addition, the legume diet included more servings of both fruits and vegetables than the prestudy diet, with the HA diet intakes between the other 2 and not significantly different from either the prestudy or legume diet. At baseline, men were 55 y of age and tended to be overweight (**Table 2**). Mean blood concentrations of lipids, lipoproteins, and biomarkers of insulin sensitivity and inflammation for the group as a whole were in the acceptable range, with the exception of LDL cholesterol (mean 3.34 mmol/L). As expected, men who were insulin resistant had higher BMI and waist circumferences and higher levels of fasting glucose, insulin, C-peptide, and triglycerides than those who were not insulin resistant. In addition, sTNFRI concentrations differed and there was a suggestion that CRP concentrations differed among the groups ($P = 0.08$).

Biomarkers of inflammation. The main effects of the 2 diets on study end points and the comparison of changes observed during each diet treatment are presented (**Table 3**). For the inflammatory markers, both diets lead to significantly reduced fasting CRP and sTNFRI and II concentrations. The greatest changes were observed for CRP; over 4 wk, plasma CRP concentrations declined 20% during the legume diet period and 18% during the HA diet period. The concentrations of the soluble receptors also improved during both diet periods, with decreases ranging from 3.7 to 5.1% for the 2 diet treatments; all changes were significant except that of sTNFRII during the legume diet period ($P = 0.06$). The beneficial effects of the 2 diets on the inflammatory markers did not significantly differ by diet treatment.

Biomarkers of insulin sensitivity. Neither the HA nor the legume diet decreased fasting insulin and C-peptide concentrations and the effects of the diets did not differ. The HA diet modestly reduced the fasting glucose concentration (2.2%; $P = 0.012$) and the legume diet increased it (1.8%; $P = 0.016$), with the effects of the diets differing significantly ($P = 0.001$).

Study endpoints stratified by insulin-resistance. There were no significant interactions between adenoma status and the diet treatments. However, the effects of the diet treatments on sTNFR I concentrations and several other biomarkers varied by IR status. Thus, we present results for the biomarkers of interest stratified by IR status in **Tables 4** [insulin sensitive (IS)] and **5** (IR). Among IS participants, fasting glucose concentrations tended to increase (1.6%; $P = 0.128$) during the legume diet period and tended to decrease (1.6%; $P = 0.177$) during the HA diet period; the effects of the 2 diets marginally differed ($P = 0.054$). The results were similar but more pronounced among IR participants; the effects of the 2 diets differed ($P = 0.005$). Fasting insulin concentrations were not significantly modified among IS participants during either diet period but decreased significantly in IR participants when they consumed the HA diet. In both cases, the results for change in insulin status did not significantly differ by experimental diet. C-peptide concentrations increased in IS participants who consumed the HA diet ($P = 0.049$). The diets differed ($P = 0.045$), with more favorable results (3% decrease vs. 10% increase) observed with the legume diet. In contrast, C-peptide concentrations changed little in IR participants and changes did not significantly differ by diet. Plasma CRP concentrations decreased in participants who consumed both diets among both strata of participants, with significant (IS HA diet) or marginally significant results observed ($P = 0.08$ for IS legume, $P = 0.10$ for IR legume, and $P = 0.07$ for IR HA diets, respectively). Decreases in mean CRP concentrations ranged from 30% for IS participants who consumed the legume diet to 11% for IR participants who consumed the legume diet. However, the comparison of the changes between the 2 diet treatments for CRP was not significant among either IS or IR participants. sTNFRI concentrations declined significantly or marginally for both diets among both strata of IR status.

TABLE 2 Baseline characteristics of study participants overall and by IR and adenoma status[1]

		IR (−)		IR (+)		
	Overall	Adenoma (−)	Adenoma (+)	Adenoma (−)	Adenoma (+)	*P*-value[2]
n	64	24	12	14	14	
Age, *y*	54.5 ± 7.8	51.7 ± 7.6[a]	58.3 ± 5.5[b]	53.4 ± 8.0[a,b]	57.6 ± 7.7[b]	0.028
BMI, *kg/m²*	28.7 ± 3.5	27.0 ± 3.5[a]	28.1 ± 2.4[a,b]	30.3 ± 3.1[b]	30.4 ± 3.5[b]	0.004
Waist circumference, *cm*	97.2 ± 9.3	92.3 ± 8.9[a]	95.1 ± 8.3[a]	101.5 ± 7.4[b]	103.2 ± 7.8[b]	0.001
SBP, *mm Hg*	123 ± 11	121 ± 7	122 ± 16	128 ± 11	123 ± 12	0.220
DBP, *mm Hg*	81 ± 7	79 ± 6	78 ± 7	84 ± 7	81 ± 5	0.095
Total cholesterol,[3] *mmol/L*	5.17 ± 0.96	5.07 ± 1.06	4.99 ± 0.72	5.41 ± 1.06	5.33 ± 0.88	0.616
HDL cholesterol,[3] *mmol/L*	1.16 ± 0.28	1.22 ± 0.34	1.22 ± 0.26	1.11 ± 0.21	1.11 ± 0.28	0.512
LDL cholesterol,[3] *mmol/L*	3.34 ± 0.83	3.31 ± 0.91	3.23 ± 0.57	3.47 ± 0.91	3.34 ± 0.93	0.931
Triglycerides,[3] *mmol/L*	1.52 ± 0.81	1.25 ± 0.62[a]	1.13 ± 0.37[a]	1.84 ± 0.95[b]	2.02 ± 0.95[b]	0.002
Glucose,[3] *mmol/L*	5.40 ± 0.49	5.19 ± 0.46	5.22 ± 0.43	5.65 ± 0.42	5.67 ± 0.47	0.002
Insulin,[3] *pmol/L*	80.6 ± 52.8	52.1 ± 17.4[a]	50.7 ± 20.1[a]	116.7 ± 69.5[b]	118.8 ± 48.6[b]	<0.001
C-peptide,[3] *nmol/L*	0.762 ± 0.364	0.563 ± 0.166[a]	0.563 ± 0.166[a]	1.060 ± 0.431[b]	0.994 ± 0.364[b]	<0.001
CRP,[3] *mg/L*	1.28 ± 1.24	0.91 ± 0.88[a]	1.51 ± 2.00[a,b]	1.61 ± 0.95[b]	1.40 ± 1.15[a,b]	0.082
sTNFRI,[3] *ng/L*	972.75 ± 166.53	920.71 ± 159.51[a]	971.75 ± 147.41[a]	937.21 ± 124.47[a]	1098.36 ± 179.30[b]	0.009
sTNFRII,[3] *ng/L*	1892.44 ± 395.24	1850.46 ± 405.69	1892.75 ± 357.09	1800.21 ± 396.75	2056.36 ± 398.99	0.334

[1] Values are the mean ± SD. Means in a row with superscripts without a common letter differ, $P < 0.05$.
[2] *P*-value represents test for overall difference across the 4 groups. Means in a row that do not share a common superscript letter are significantly different ($\alpha = 0.05$).
[3] Serum was used for lipids, and C-peptide and plasma for CRP, insulin, glucose, and sTNFRI and II.

TABLE 3 Effects of the legume and HA diets on biomarkers of IR and inflammation in men at risk for colorectal cancer[1]

	Baseline	Δ Legume[2]	*P*-value	Δ Am[2]	*P*-value	Δ Legume–Δ Am[2]	*P*-value
Glucose, *mmol/L*	5.40 ± 0.49	0.10 ± 0.04	0.016	−0.12 ± 0.05	0.012	0.22 ± 0.06	0.001
Insulin, *pmol/L*	80.6 ± 52.8	−2.3 ± 2.6	0.408	−7.2 ± 3.8	0.443	4.9 ± 4.3	0.955
C-peptide, *nmol/L*	0.762 ± 0.364	−0.021 ± 0.024	0.407	−0.001 ± 0.026	0.605	−0.02 ± 0.033	0.332
CRP, *mg/L*	1.28 ± 1.24	−0.259 ± 0.129	0.018	−0.234 ± 0.101	0.007	−0.025 ± 0.167	0.930
sTNFRI, *ng/L*	972.75 ± 166.53	−35.85 ± 12.21	0.005	−42.95 ± 11.91	0.001	7.09 ± 17.81	0.692
sTNFRII, *ng/L*	1892.44 ± 395.24	−71.09 ± 37.06	0.060	−95.94 ± 22.25	<0.001	24.85 ± 44.57	0.579

[1] Values are the mean ± SEM, $n = 64$. Statistical significance was set at $\alpha = 0.05$.
[2] Δ Legume = change over the legume diet; Δ Am = change over the HA diet, Δ Legume − Δ Am = change between the 2 diets.

Decreases in the sTNFRII concentration were apparent only for the HA diet period among both strata; the comparisons of the changes for either receptor between the 2 diets was not significant among either IS or IR participants.

Discussion

This controlled feeding study explored the effects of a low-GI, high-legume diet on biomarkers of IR and inflammation among a population of older men at risk for colorectal cancer. Previous analyses conducted by our group within the PPT (5) suggested that legume consumption may protect against colorectal adenoma recurrence. In the present study, we observed that both the control (HA) and high-legume diets had favorable effects on many endpoint biomarkers that have been associated with incidence of colorectal cancer and adenomas compared with the participants' usual diets. For example, in the main effects analysis (Table 3), both test diets had favorable effects on fasting CRP and sTNFRI and II in comparison to the participants' own usual diets. This may be explained in part by comparison of the nutrient profiles of the participants' prestudy diets to the 2 experimental diets, which demonstrates that although some differences did not reach significance, both test diets tended to be more healthful than the participants' typical diets (Table 1). For example, both experimental diets had higher concentrations of vitamin C, known to be associated with decreased levels of inflammatory markers (30–33). Dietary cholesterol was also higher among participants' usual diets (125 mg/1000 kcal) compared with the HA (98 mg/1000 kcal) and the high-legume diets (70 mg/1000 kcal), although the results achieved significance only for the pairwise comparison between the prestudy and high-legume diets. When changes in concentrations of fasting biomarkers due to the 2 experimental diets were compared, the high-legume and HA diets did not differ. These results did not support our hypothesis that the legume-enriched diet would lead to more favorable changes in the biomarkers of interest compared with the HA diet. However, the results provide support for the role of healthful dietary changes, including a high-legume diet, in improving biomarkers of IR and inflammation independent of weight loss.

In contrast to the fasting blood results, following a 2-h challenge breakfast with each of the experimental diets, the mean increase in insulin (48 pmol/L) and C-peptide concentrations (0.50 nmol/L) during the legume diet period was less than during the HA diet period (193.9 pmol/L and 1.308 nmol/L; $P < 0.001$) (data not shown). We collected only fasting and 2-h postprandial blood samples during the challenge and do not have additional time points for comparison.

Although this was intentional, our efforts to keep the participants' weights stable across the diet periods may have limited our ability to detect differences in biomarkers between the 2 dietary interventions. The participants' weights were closely monitored during the study, so that if weight changed, the energy content of the diet was adjusted for weight maintenance. While consuming the legume diet in particular, men reported feeling very full. If the protective effect observed with high legume consumption in the PPT was due to weight loss facilitated by legume consumption, which then resulted in favorable effects on IR and inflammation, then weight maintenance in the current study may have kept the biomarkers of interest at relatively stable levels. An additional hypothesis being investigated in an optional 3rd 4-wk dietary treatment with a subset of the original participants ($n = 45$) is that the legume-enriched diet will result in changes in circulating levels of satiety hormones, which will tend to reduce body weight once weight maintenance is no longer required. The effect of the legume diet on weight loss is particularly important, because obesity is a risk factor for colorectal cancer, IR, and type 2 diabetes. It is also

TABLE 4 Effects of the legume and HA diets on biomarkers of IR and inflammation among IS men[1]

IR⁻ (IS)	Baseline	Δ Legume[2]	*P*-value	Δ Am[2]	*P*-value	Δ Legume−Δ Am[2]	*P*-value
Glucose, *mmol/L*	5.20 ± 0.44	0.08 ± 0.05	0.128	−0.08 ± 0.06	0.177	0.17 ± 0.08	0.054
Insulin, *pmol/L*	51.7 ± 17.9	0.8 ± 3.5	0.947	1.3 ± 4.8	0.233	−0.6 ± 5.6	0.387
C-peptide, *nmol/L*	0.553 ± 0.166	−0.017 ± 0.033	0.402	0.056 ± 0.033	0.049	−0.07 ± 0.043	0.046
CRP, *mg/L*	1.11 ± 1.36	−0.34 ± 0.17	0.082	−0.18 ± 0.14	0.039	−0.16 ± 0.22	0.920
sTNFRI, *ng/L*	937.72 ± 155.39	−31.60 ± 16.38	0.058	−37.04 ± 15.73	0.022	5.44 ± 24.32	0.824
sTNFRII, *ng/L*	1864.56 ± 385.54	−67.88 ± 50.02	0.180	−58.26 ± 28.66	0.046	−9.63 ± 60.12	0.873

[1] Values are the mean ± SEM, $n = 36$. Statistical significance was set at $\alpha = 0.05$.
[2] Δ Legume = change over the legume diet; Δ Am = change over the HA diet, Δ Legume − Δ Am = change between the 2 diets.

TABLE 5 Effects of the legume and HA diets on biomarkers of IR and inflammation among IR men[1]

IR+	Baseline	Δ Legume[2]	*P*-value	Δ Am[2]	*P*-value	Δ Legume–Δ Am[2]	*P*-value
Glucose, *mmol/L*	5.66 ± 0.44	0.12 ± 0.06	0.055	−0.16 ± 0.07	0.023	0.28 ± 0.10	0.005
Insulin, *pmol/L*	117.8 ± 58.9	−6.3 ± 4.0	0.240	−18.5 ± 5.6	0.011	12.2 ± 6.5	0.271
C-peptide, *nmol/L*	1.024 ± 0.384	−0.03 ± 0.036	0.762	−0.076 ± 0.036	0.140	0.046 ± 0.05	0.408
CRP, *mg/L*	1.51 ± 1.04	−0.16 ± 0.20	0.108	−0.30 ± 0.16	0.078	0.15 ± 0.26	0.985
sTNFRI, *ng/L*	1017.79 ± 172.25	−34.35 ± 18.51	0.068	− 43.51 ± 18.74	0.024	9.15 ± 28.11	0.746
sTNFRII, *ng/L*	1928.29 ± 411.64	−75.73 ± 56.67	0.186	−148.39 ± 33.78	<0.001	72.65 ± 68.73	0.295

[1] Values are the mean ± SEM, $n = 28$. Statistical significance was set at $\alpha = 0.05$.
[2] Δ Legume = change over the legume diet; Δ Am = change over the HA diet, Δ Legume − Δ Am = change between the 2 diets.

possible that the protective effects associated with legume consumption in the PPT were due to other anticarcinogenic components in beans such as phenolic compounds, saponins, and protease inhibitors acting through mechanisms unrelated to insulin sensitivity and inflammation.

Overall, the limited numbers of studies that have evaluated the role of GI and GL on biomarkers of insulin sensitivity and inflammation have reported conflicting results. Some of the conflicting results may be due to differences in population characteristics (e.g. BMI, age, gender, smoking status), the method of dietary data collection (24-h recall, food record, food frequency), the range of GI and GL values observed, the variability and range (e.g. risk level) of the biomarkers evaluated, other study design-related issues (e.g. weight maintenance vs. loss and test diet macronutrient profiles). In a cross sectional study including 582 participants of both genders with diet assessed by repeated 24-h recalls, Griffith et al. (34) found no association between either GI or GL and CRP. These results were unexpected, given reports of significant inverse associations between dietary fiber with CRP, interleukin-6, and sTNFRII in this population (35,36), and inverse associations between GI/GL and CRP in 2 other cross-sectional investigations (24,25). These 2 investigations both included only women and used an FFQ for dietary assessment. In addition, the Levitan (25) study was much larger ($n = 18{,}137$) than any other investigation, which may have contributed to their significant findings.

It is well recognized that dietary modification can influence glycemic response. Most well-controlled, short-term clinical studies of low-GI diets have demonstrated improvements in insulin sensitivity and B-cell function (37–41) and a meta-analysis of 14 randomized crossover or parallel arm studies concluded that low-GI diets reduced glycated proteins 7.4% more than did high-GI diets (42). Unexpected results were observed in a recently published 4-mo trial among 34 participants with impaired glucose tolerance. Wolever et al. (38) measured plasma glucose and insulin levels when participants were fasting and at multiple nonfasting time points during the day. Their results demonstrated that a low-GI diet (GI 53.1) reduced postprandial glucose concentrations compared with a high-GI diet (GI 61.1); however, mean plasma insulin concentrations were 20% higher on the low-GI diet compared with the high-GI diet. The investigators speculated that decreases in plasma insulin concentrations in the high-GI group may be a result of deteriorating B-cell function within their population. An earlier study by Kiens and Richter (43) comparing low- and high-GI diets in a crossover design with 7 healthy, young men, reported that initially blood glucose and insulin levels throughout the day were reduced by the low-GI (GI 66) compared with the high-GI (GI 90) diet ($P < 0.05$); however, after 1 mo, the differences between the 2 diets were attenuated and were no longer significant.

The results of clinical trials designed to evaluate the effects of dietary interventions altering the quantity or quality of dietary carbohydrate on inflammatory markers are inconsistent. The Canadian Trial of Carbohydrates in Diabetes (44) recently published results from a 1-y controlled trial of diets with varying GI among 162 participants with type 2 diabetes. They tested 3 diets with participants of both genders: high-GI/high-carbohydrate, low-GI/high-carbohydrate, and low-carbohydrate/high-monounsaturated fat with carbohydrate as 47, 52, and 39% of total energy, respectively. The GI of the respective diets was determined by 3-d food records collected 5 times throughout the intervention period and estimated at 63, 55, and 59. Similar to our results, the Canadian Trial of Carbohydrates in Diabetes investigators observed that fasting plasma glucose concentrations after 1 y were higher among participants on the low-GI compared with the high-GI diet and that fasting plasma insulin levels did not differ. Postprandial glucose levels following a typical breakfast for each diet were lower on the low-GI diet. Lastly, in contrast to our results, mean CRP concentrations on the low-GI diet were 30% less than the high GI diet ($P = 0.0078$) after 1 y.

In summary, the results from this randomized, controlled, feeding trial comparing a low-GI, legume-enriched diet to a HA diet were not supportive of our original hypothesis that the high-legume, low-GI, high-fiber diet would improve fasting biomarkers of insulin sensitivity and inflammation after 4 wk compared with a HA diet. Compared with the participants' usual diets, both diets favorably improved fasting biomarkers of inflammation. Our study did support the concept that a high-legume diet reduced the rate of the absorption of the carbohydrates and lowered the postprandial glycemic and insulinemic responses leading to a reduced level of C-peptide. These postprandial results were limited in scope and should be interpreted as such. It is possible that a longer intervention period or a larger dissimilarity in experimental diets is needed to observe differences in fasting biomarkers or that the link between colon cancer incidence and increased CRP levels might be a reflection of the link between a concomitant increase in CRP concentrations with obesity and increased colorectal cancer risk. This possibility and whether weight loss contributed to the risk reduction in the highest quartile of legume consumption in the PPT need further study.

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