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Effects of lupin kernel flour–enriched bread on blood pressure: a controlled intervention study[1–3]

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ABSTRACT

Background: Available data suggest that substitution of refined carbohydrate in the diet with protein and fiber may benefit blood pressure. Lupin kernel flour is high in protein and fiber and low in carbohydrate.

Objective: Our objective was to determine the effects on blood pressure of a diet moderately higher in dietary protein and fiber achieved by substituting lupin kernel flour for wheat flour in bread.

Design: Overweight and obese men and women (n = 88) were recruited to a 16-wk parallel-design study. Participants were randomly assigned to replace 15–20% of their usual daily energy intake with white bread (control) or lupin kernel flour–enriched bread (lupin). Measurements, including 24-h ambulatory blood pressure, were taken at baseline and 16 wk.

Results: Seventy-four participants (37 per group) completed the intervention. Baseline mean (±SD) systolic/diastolic blood pressures were 122.1 ± 9.6/70.8 ± 7.2 mm Hg (control) and 120.1 ± 9.5/71.2 ± 5.9 mm Hg (lupin). For lupin relative to control, the estimated mean (95% CI) net differences in protein, fiber, and carbohydrate intakes during the intervention were 13.7 g/d (95% CI: 2.3, 25.0 g/d), 12.5 g/d (95% CI: 8.8, 16.2 g/d), and −19.9 g/d (95% CI: −45.2, 5.5 g/d), respectively. Differences in systolic blood pressure, diastolic blood pressure, pulse pressure, and heart rate were −3.0 mm Hg (95% CI: −5.6, −0.3 mm Hg; P = 0.03), 0.6 mm Hg (95% CI: −1.0, 2.2 mm Hg; P = 0.47), −3.5 mm Hg (95% CI: −5.3, −1.8 mm Hg; P < 0.001), and 0.0 beats/min (95% CI: −1.7, 1.7 beats/min; P = 0.99), respectively.

Conclusions: Increasing protein and fiber in bread with lupin kernel flour may be a simple dietary approach to help reduce blood pressure and cardiovascular risk. This trial was registered at the Australian New Zealand Clinical Trials Registry at http://www.anzctr.org.au/trial_view.aspx?ID=1014 as ACTRN12606000034538 on 25 January 2006. *Am J Clin Nutr* 2009;89:766–72.

INTRODUCTION

Lupin kernel flour is a novel food ingredient derived from the endosperm of lupin, a grain legume. It contains 40–45% protein, 25–30% fiber, and negligible sugar and starch (1). It can be incorporated into high carbohydrate foods, resulting in significant increases in protein and fiber, reductions in refined carbohydrate, and little change in product acceptability (2).

Increasing protein at the expense of refined carbohydrate in the diet may benefit blood pressure. An inverse association between estimated protein intake and blood pressure was reported in many cross-sectional population studies (3–5). In randomized controlled trials, lower blood pressure with protein, in comparison to carbohydrate, is also a consistent finding (6–10). Differences in systolic blood pressure of 1.4 (9) to 5.9 mm Hg (6) were shown with higher protein and lower carbohydrate intakes, ranging from 25 to 66 g/d. Both protein and carbohydrate may play a role in influencing blood pressure in these studies. A meta-analysis of randomized controlled trials comparing carbohydrate with monounsaturated fat found that a higher intake of carbohydrate of ≈55 g/d resulted in higher systolic and diastolic blood pressures of 1.3 and 0.9 mm Hg, respectively (11).

An increased intake of dietary fiber may also lower blood pressure. Many randomized controlled trials have now investigated the effects of increasing fiber intake on blood pressure. Meta-analyses of these trials showed that an increase in fiber intake of 10–15 g/d was associated with falls in systolic and diastolic blood pressures of ≈1–1.5 mm Hg (12, 13). Soluble fiber may be more effective than insoluble fiber (12). In addition, more substantial decreases in systolic and diastolic blood pressures were observed in trials of >8-wk duration (3.1 and 2.6 mm Hg; systolic and diastolic blood pressures, respectively) and in hypertensive subjects (6.0 and 4.2 mm Hg; systolic and diastolic blood pressures, respectively) (13).

Increasing both protein and fiber intakes, at the expense of refined carbohydrate, may benefit blood pressure. We have previously shown that the combined effects of an additional intake of 66 g/d dietary protein and 15 g/d soluble fiber resulted in significant additive effects to lower systolic blood pressure by ≈10 mm Hg. This effect was observed against the background of a low-protein, high-carbohydrate, and low dietary fiber diet in hypertensive persons (6). However, this was achieved with the use of dietary supplements, and such large increases in protein intake in particular may be difficult to achieve and sustain with foods in the wider population.

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Our aim therefore was to determine the effects on blood pressure of a diet moderately higher in dietary protein and fiber. The differences in nutrient composition was achieved by substituting wheat flour with lupin kernel flour in bread.

SUBJECTS AND METHODS

Participants

Overweight and obese men and women were targeted for recruitment to this trial. Excess body fat, now prevalent in many populations, can cause a cluster of metabolic disorders, one of which is elevated blood pressure (14). Participants had a body mass index (BMI; in kg/m^2) between 25 and 35 at screening. Nonsmoking men and women aged between 20 and 70 y were recruited from the general population with the use of newspaper advertisements. Exclusion criteria included history of cardiovascular or peripheral vascular disease; diabetes; fasting plasma glucose concentrations ≥5.6 mmol/L; history of asthma, renal disease, liver disease, or gout; psychiatric illness; history of major gastrointestinal problems; other major illnesses such as cancer; uncontrolled hypertension (systolic blood pressure >150 mm Hg or diastolic blood pressure >95 mm Hg); use of >2 antihypertensive agents; a change in antihypertensive, lipid-lowering, or other medication within the previous 3 mo; women who were pregnant or intended to become pregnant; history of food allergies; current weight loss, and alcohol intake >200 g/wk for women and >300 g/wk for men. The study was approved by the University of Western Australia Human Ethics Committee. All participants provided written informed consent. Procedures followed were in accordance with institutional guidelines.

Study design

A total of 88 participants were recruited to a 16-wk randomized, controlled parallel-designed trial. Participants were randomly assigned with the use of computer-generated random numbers concealed in opaque envelopes to 1 of 2 groups: control or lupin. Participants maintained their usual diet, physical activity, and medication regimen during a baseline period of 2 wk, which preceded random assignment. Both groups were then required to replace ≈15–20% of their usual daily energy intake with bread, either control or lupin, for 16 wk. For an energy intake of 9 MJ/d, this would equate to ≈4 × 40-g slices of bread per day. Participants were not blinded to their treatment allocation because of obvious differences in color and texture of the 2 bread types.

The formulation of the 2 breads was described in detail previously (2). Briefly, a standard white bread, with small modifications to the recipe, was used as the control. The lupin bread was formulated by substituting 40% of the wheat flour usually present in the white bread with lupin kernel flour. Adjustments to the recipes were made to match breads for total fat; saturated, monounsaturated, and polyunsaturated fats; protein derived from wheat; and sodium. Compared with the control bread, the lupin bread was higher in protein and fiber derived from lupin kernel flour and lower in wheat-derived carbohydrate. The nutrient composition of the breads was analyzed by BRI Australia Ltd (North Ryde, Australia) (**Table 1**). On average, each slice of lupin bread was heavier than each slice of white bread by ≈15–20%. Thus, total energy content for a specific number of slices of each bread type was similar. No significant difference was observed in overall palatability of the 2 breads when consumed toasted or not toasted as sandwiches (2).

The bread was provided to participants and replaced their usual bread intake and where necessary other carbohydrate-rich foods such as pasta, rice, and breakfast cereals. A dietitian counseled the participants at baseline on how to incorporate the bread into their usual diet. The participants were instructed not to alter their food intake, apart from prescribed changes, and to maintain their usual level of physical activity. Medication remained as prescribed and did not change throughout the intervention. All measurements were performed at baseline and at completion of the 16-wk intervention.

A dietitian counseled the participants every 2 wk throughout the intervention to ensure achievement of the bread incorporation and maintenance of usual lifestyle. Every 2 wk, the participants were supplied with enough fresh sliced bread, which was then used fresh or was frozen before use, during the following 2 wk. Bread was provided based on usual energy intake assessed at baseline to supply 15–20% of the participant's daily energy intake. The aim was to achieve mean between-group differences in protein, carbohydrate, and fiber intakes of ≈15 g/d, −20 g/d, and 13 g/d, respectively, for lupin relative to control.

Dietary assessment

Dietary intake was assessed with the use of a modified diet history questionnaire at baseline and at the end of 16 wk (15). Participants were asked to describe their usual daily eating pattern, including variations. Portion size was estimated with the use of food models. This questionnaire was administered by a qualified dietitian. The information collected from the questionnaire was analyzed with the use of Foodworks Professional 2007 (Xyris, Highgate Hill, Australia) to determine average daily energy and macronutrient intakes. Compliance with the bread intake was assessed with the use of a daily bread intake record in which participants recorded the number of slices consumed each day throughout the study. Every 2 wk, the dietitian monitored bread intake; if bread intake was outside the prescribed amount, participants were counseled on how the correct number of slices could be incorporated in their diet. Participants who were unable to consume ≥50% of the prescribed quantity of bread were withdrawn from the study. Diet and lifestyle were also monitored by the dietitian at these visits with the use of questions that asked about

TABLE 1
Nutrient composition of the control and lupin breads

	Control (white bread)		Lupin (lupin-enriched bread)	
	g/1000 kJ	*g/100 g*	*g/1000 kJ*	*g/100 g*
Bread weight	90	—	113	—
Water	32.9	36.6	48.6	43.0
Fat	4.1	4.6	4.1	3.6
Protein	8.6	9.6	17.9	15.8
Fiber	2.4	2.7	10.7	9.5
Carbohydrate	40.7	45.2	28.1	24.9
Ash	1.5	1.7	2.4	2.1
Sodium	0.54	0.60	0.54	0.48

any recent changes. If changes to diet or lifestyle were reported, advice was provided on how these could be adjusted. If these changes could not be rectified or if they resulted in an inability to commit to the time requirements of the study, the participant was withdrawn. Body weight was also measured at these visits.

Blood pressure assessment

Twenty-four-hour ambulatory blood pressure was measured at baseline and at the end of 16 wk. Measurements were performed with the use of Spacelab monitors (Spacelabs 90207; Medtel Pty Ltd, West Leederville, WA), programmed to take a reading every 20 min while the participant was awake and every 30 min while asleep. The monitor was fitted to the nondominant arm ≈2.5 cm above the antecubital fossa by a trained researcher. Adult- or large adult–sized cuffs were used, depending on the upper arm circumference assessed at baseline. Participants were requested to continue with their normal routine and to avoid any vigorous exercise. They also completed a diary to record the time and the activity they were undertaking at the time of each measurement and to record waking and sleeping times. Measurements showing an error code or pulse pressure <20 mm Hg were excluded from the analysis. Blood pressure traces were regarded as being complete if >80% of the recordings were valid. Complete ambulatory blood pressure data were available for all 74 participants who completed the trial.

Biochemistry

At baseline and at 16 wk, blood samples were taken after a 12-h fast from the antecubital fossa vein, and a 24-h urine collection was performed. For the 24-h urine samples, participants were instructed to start the 24-h period on waking in the morning immediately after emptying their bladder. All urine was then collected for 24 h, with the final sample in the morning on waking the next day. All routine biochemical analyses were performed by the Department of Clinical Biochemistry at Royal Perth Hospital, Western Australia. Blood samples were centrifuged at 3000 rpm at 4°C for 10 min and stored at −80°C until analysis. Aliquots of urine were also stored at −80°C until analysis. All samples were run in a single batch to reduce variability. Serum and urinary creatinine concentrations were measured with the use of kinetic colorimetric tests (Roche, Indianapolis, IN) with an automated analyzer (Roche Hitachi 917). The CV of the assay was <6%. Serum and 24-h urinary sodium and potassium concentrations were measured with the use of an ion-selective electrode with an automated analyzer (Roche Hitachi 917). The CVs of the sodium and potassium assays were <2%.

Statistical analysis

Statistical analyses were performed with the use of SPSS 15.0 software (SPSS Inc, Chicago, IL) or SAS 8.2 software (SAS Institute, Cary, NC). For descriptive data, including baseline and 16-wk measurements, results are presented as mean ± SD. The baseline-adjusted end of intervention values and between-group differences are presented as mean (95% CI). $P < 0.05$ was the level of significance in 2-tailed testing. The sample for this study was calculated on the primary outcome of 24-h ambulatory systolic blood pressure. With $\alpha = 0.05$, 40 participants per group provided >90% power to detect a difference between groups of 3 mm Hg. This power calculation is based on a within-group SD at both baseline and after intervention of 15 mm Hg and assumed a within-subject correlation for baseline and postintervention measurements of 0.5. We aimed to recruit 90 participants to the study to allow for an estimated 10% withdrawal rate over the 16-wk intervention. The primary analysis included participants who completed the intervention. At baseline, characteristics of participants in the 2 groups were compared with the use of the independent-sample t test and the chi-square test for categorical variables. General linear models (analysis of covariance) were used to assess baseline-adjusted end-of-intervention between-group differences. The baseline-adjusted end-of-intervention between-group differences in ambulatory blood pressure and heart rate were analyzed with random-effects models in SAS with the use of PROC MIXED (SAS Institute). In the random-effects models, the subject was treated as the random effect, which accounts for correlated error structures, and the treatment (control or lupin) was treated as the fixed effect. Between-group differences were also adjusted for potential confounding factors, including age, sex, changes in weight, alcohol intake, magnesium intake, and urinary sodium and potassium excretions, which were included as covariates in the random-effects models. Separate subgroup analyses of blood pressure differences were also performed with random-effects models in SAS with the use of the World Health Organization–International Society of Hypertension upper limit for normal 24-h ambulatory blood pressure of 125/80 mm Hg (16).

RESULTS

Recruitment

The study was performed between June 2006 and July 2007. The trial profile, with the number of persons screened, excluded, and randomly assigned, and the number of persons that withdrew from the study is presented in **Figure 1**. A total of 88 participants were randomly assigned to the trial. In the control group 11 participants withdrew: 7 because they were unable to consume the required quantity of bread, 3 because of an inability to commit to time requirements, and 1 because of moving to another location. In the lupin group, 3 participants withdrew: 1 because of an inability to consume the required quantity of bread, 1 because of an inability to commit to time requirements, and 1 because of an unrelated medical problem requiring a change in medication. No adverse effects of eating either the white or the lupin bread were reported during the 16-wk study. The study was conducted with the use of 2 cohorts. The first cohort completed the study between June and December 2006, and the second completed the study between January and July 2007. In the first cohort, participants were randomly assigned to the control or lupin group in a ratio of 1:1. Because of a larger number of withdrawals in the control group in the first cohort, in the second cohort participants were randomly assigned to the control or lupin group in a ratio of 3:2. Given that the primary analysis was to include only those participants completing the study, the objective was to achieve similar numbers in each group. For both the control and lupin groups, no significant differences in sex, age, BMI, energy intake, systolic and diastolic blood pressures, and heart rate were observed at baseline between those participants who completed the intervention and those who withdrew.

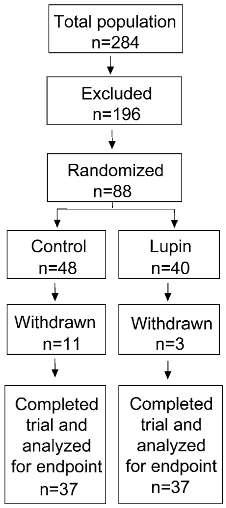

FIGURE 1. Flow chart of participants at each stage of the trial.

Baseline characteristics

A total of 74 participants completed the trial. Baseline characteristics of the participants are presented in **Table 2**. The groups were well matched for use of lipid-lowering and antihypertensive medications. In the control group, 8 were taking statins, 2 were taking aspirin, 4 were taking angiotensin-converting enzyme inhibitors, 4 were taking angiotensin II receptor blockers, 1 was taking calcium channel blockers, and 4 were taking diuretics. In the lupin group, 4 were taking statins, 1 was taking aspirin, 1 was taking angiotensin-converting enzyme inhibitors, 4 were taking angiotensin II receptor blockers, 2 were taking β-blockers, and 2 were taking diuretics. The estimated mean ($\pm$SD) energy intakes from all bread at baseline were 1182 $\pm$ 675 kJ/d and 1251 $\pm$ 925 kJ for the control and lupin groups, respectively. This equates to $\approx$3 $\times$ 40-g slices/d. No significant difference in any of these variables was observed between the groups at baseline.

Body weight, energy and nutrient intakes, and serum and urinary analytes

During the intervention, the mean ($\pm$SD) energy intakes from the assigned breads were 1551 $\pm$ 194 kJ/d and 1638 $\pm$ 248 kJ/d in the control and lupin groups, respectively, which were not significantly different. Approximately 75% of the energy from the assigned breads replaced energy from usual bread intake. On the basis of this bread intake alone, for lupin compared with control, this would equate to higher lupin-derived protein and fiber of $\approx$16 g/d and 14 g/d, respectively, and lower wheat-derived carbohydrate by $\approx$17 g/d. Although participants were instructed not to alter their diet apart from the prescribed changes, it is important to take into account the entire diet when assessing differences in nutrient intakes.

Body weight, energy and nutrient intakes, and urinary analytes at baseline and at the end of intervention are presented in **Table 3**. No significant between-group differences were observed at baseline. Compared with the control group, there was a higher intake of protein (13.7 g/d; 95% CI: 2.3, 25.0) and fiber (12.5 g/d; 95% CI: 8.8, 16.2). Although the observed difference in carbohydrate was close to the expected difference of 20 g/d (−19.9 g/d; 95% CI: −45.2, 5.5), this did not reach significance. With the exception of magnesium, intakes of other nutrients, total energy, and measurements of body weight and urinary and serum analytes were not significantly different between groups.

Blood pressure

The mean 24-h, awake and asleep systolic and diastolic blood pressures were not statistically different between the groups at baseline (Table 2). The baseline-adjusted end-of-intervention

TABLE 2
Baseline characteristics of participants in the control and lupin groups

	Control (n = 37)	Lupin (n = 37)	P[1]
Descriptive data			
Men/women (n)	14/23	12/25	0.81
Age (y)	56.8 ± 8.5[2]	59.0 ± 7.4	0.25
BMI (kg/m^2)	30.5 ± 3.4	30.6 ± 3.6	0.91
Blood pressure and heart rate			
24-h systolic blood pressure (mm Hg)	122.1 ± 9.6	120.1 ± 9.5	0.39
24-h diastolic blood pressure (mm Hg)	70.8 ± 7.2	71.2 ± 5.9	0.80
24-h pulse pressure (mm Hg)	51.3 ± 8.5	49.0 ± 6.4	0.19
24-h heart rate (beats/min)	72.3 ± 5.8	70.9 ± 7.9	0.14
Awake systolic blood pressure (mm Hg)	127.7 ± 9.6	125.6 ± 10.0	0.36
Awake diastolic blood pressure (mm Hg)	75.2 ± 7.4	76.0 ± 5.8	0.62
Awake pulse pressure (mm Hg)	52.5 ± 8.8	49.7 ± 6.9	0.13
Awake heart rate (beats/min)	76.0 ± 5.8	74.8 ± 8.7	0.48
Asleep systolic blood pressure (mm Hg)	109.7 ± 11.9	108.2 ± 10.7	0.57
Asleep diastolic blood pressure (mm Hg)	61.1 ± 8.6	60.8 ± 7.8	0.84
Asleep pulse pressure (mm Hg)	48.6 ± 8.9	47.5 ± 6.2	0.52
Asleep heart rate (beats/min)	64.2 ± 6.9	62.6 ± 7.4	0.34

[1] P value for between-group difference analyzed with the independent samples t test and the chi-square test for categorical variables.
[2] Mean ± SD (all such values).

TABLE 3
Body weight, energy and nutrient intakes, and urinary and serum analytes of the participants at baseline and the end of the intervention

	Control (n = 37)		Lupin (n = 37)		
Variable	Baseline	End of intervention	Baseline	End of intervention	P[1]
Body weight and energy intake					
Body weight (kg)	86.6 ± 14.5[2]	86.3 ± 14.9	85.8 ± 11.2	85.1 ± 11.1	0.45
Energy intake (mJ/d)	9.67 ± 2.23	8.88 ± 2.13	9.28 ± 2.09	8.23 ± 2.06	0.28
Nutrient intakes					
Total fat (% of energy)	32.1 ± 7.96	34.0 ± 8.54	31.7 ± 8.15	30.9 ± 7.10	0.09
Saturated fat (% of energy)	12.8 ± 3.71	11.4 ± 3.85	13.8 ± 4.50	11.9 ± 3.40	0.75
Monounsaturated fat (% of energy)	11.5 ± 3.62	13.1 ± 4.48	10.9 ± 3.43	11.5 ± 3.07	0.11
Polyunsaturated fat (% of energy)	5.06 ± 2.07	6.77 ± 3.30	3.98 ± 1.84	4.94 ± 2.40	0.06
Protein (% of energy)	21.4 ± 3.94	20.3 ± 4.26	21.1 ± 4.65	24.3 ± 4.99	0.002
Protein (g/d)	120.6 ± 33.0	104.6 ± 27.9	114.5 ± 36.9	115.5 ± 30.3	0.02
Carbohydrate (% of energy)	41.3 ± 7.53	40.1 ± 8.30	42.7 ± 7.50	38.9 ± 7.99	0.30
Carbohydrate (g/d)	234.7 ± 69.8	210.8 ± 68.8	233.2 ± 64.8	190.1 ± 64.7	0.12
Fiber (g/d)	29.2 ± 9.18	24.2 ± 8.89	28.9 ± 12.3	36.5 ± 9.80	<0.001
Alcohol (g/d)	11.3 ± 16.5	9.92 ± 16.8	8.63 ± 12.2	8.38 ± 10.7	0.99
Sodium (g/d)	2.63 ± 1.17	2.44 ± 1.01	2.49 ± 1.27	2.08 ± 0.93	0.14
Potassium (g/d)	4.17 ± 1.01	3.71 ± 0.84	4.28 ± 1.22	4.01 ± 0.94	0.16
Magnesium (mg/d)	438 ± 97	381 ± 97	448 ± 147	425 ± 89	0.04
Calcium (mg/d)	1308 ± 579	1009 ± 0.84	1529 ± 694	1017 ± 333	0.65
Urinary and serum analytes					
24-h creatinine excretion (mmol/d)	12.1 ± 4.7	11.4 ± 3.5	12.3 ± 3.4	11.9 ± 3.2	0.54
Sodium excretion (mmol/mmol creatinine)	14.1 ± 5.53	14.1 ± 4.80	13.6 ± 3.35	14.4 ± 3.90	0.66
Potassium excretion (mmol/mmol creatinine)	6.94 ± 2.26	6.77 ± 2.33	6.64 ± 1.87	7.23 ± 1.90	0.11
Serum creatinine (μmol/L)	73.2 ± 14.1	72.9 ± 14.8	72.7 ± 11.4	70.3 ± 12.5	0.12
Serum sodium (mmol/L)	140.4 ± 1.5	140.0 ± 1.7	140.0 ± 1.5	140.0 ± 1.7	0.90
Serum potassium (mmol/L)	4.11 ± 0.21	4.12 ± 0.22	4.07 ± 0.32	4.08 ± 0.29	0.57

[1] P value is for baseline-adjusted end-of-intervention differences analyzed with general linear models.
[2] Mean ± SD (all such values).

blood pressure and heart rate and between-group differences are presented in **Table 4**. The 24-h systolic blood pressure and pulse pressure were significantly lower in the lupin group by 3.0 and 3.5 mm Hg, respectively, than in the control group. Diastolic blood pressure and heart rate were not significantly different between groups. The magnitude of the differences in awake and asleep systolic blood pressure and pulse pressure were similar to those observed over 24 h (Table 4). The observed differences in 24-h systolic blood pressure and pulse pressure were independent of age, sex, and changes in weight, alcohol intake, magnesium intake, and urinary sodium or potassium excretion.

Separate subgroup analyses were also performed, which included participants with above optimal (>125 or 80 mm Hg) and optimal (≤125 or 80 mm Hg) 24-h systolic and diastolic blood pressure. For participants with above optimal blood pressure, the differences in 24-h systolic and diastolic blood pressures for lupin relative to control were as follows: −4.1 mm Hg (95% CI: −7.6, 0.6; $P = 0.02$) and −1.0 mm Hg (95% CI: −3.8, 1.8; $P = 0.48$), respectively. The corresponding differences in those with optimal blood pressure were −2.2 (95% CI; −4.9, 0.6; $P = 0.12$) and 1.0 mm Hg (95% CI: −0.8, 2.8; $P = 0.27$).

An intention-to-treat analysis was also performed with data from all 88 participants randomly assigned to the study. The mean baseline-adjusted end-of-intervention between-group differences in 24-h systolic blood pressure (−2.6 mm Hg; 95% CI: −4.7, −0.6; $P = 0.01$), diastolic blood pressure (0.8 mm Hg; 95% CI: −0.5, 2.2;, $P = 0.22$), pulse pressure (−3.4 mm Hg; 95% CI: −4.8, 2.1; $P < 0.0001$), and heart rate (−0.9 beats/min; 95% CI: −2.3, 0.6; $P = 0.23$) were similar to the observed differences in the 74 participants who completed the study.

DISCUSSION

Our aim was to determine the effects on blood pressure of a diet moderately higher in dietary protein and fiber. Effects of regular consumption of lupin kernel flour–enriched bread, which is higher in protein and fiber and lower in refined carbohydrate, were compared with a standard white bread. Consumption of the lupin bread resulted in higher intakes of protein and fiber by ≈14 g/d and 13 g/d, respectively. It also resulted in a lower 24-h systolic blood pressure of 3.0 mm Hg and a lower 24-h pulse pressure of 3.5 mm Hg. Diastolic blood pressure and heart rate were not significantly different between treatments.

Available evidence suggests that substitution of refined carbohydrate in the diet with protein and fiber may benefit blood pressure (7–10). This has yet to be translated into effective and widely applicable approaches for the prevention and treatment of elevated blood pressure. Possible reasons for this are that large differences in protein intake may be difficult to achieve and sustain, and advice to increase protein intake could result in an increase in saturated fat intake from animal sources of protein. In addition, advice targeting a range of high-fiber foods may not lead to a long-term increase in fiber intake. Our approach was to alter the nutrient composition of a single staple food, bread. Bread is a staple food in many populations and

TABLE 4

Mean baseline-adjusted, end-of-intervention ambulatory blood pressures and heart rate and between-group differences of participants in the control and lupin groups

	Control (n = 37)[1]	Lupin (n = 37)[1]	Difference[2]	P[3]
24-h Ambulatory blood pressure and heart rate				
Systolic blood pressure (mm Hg)	123.0 (121.1, 124.9)	120.0 (118.1,121.9)	−3.0 (−5.6, −0.3)	0.03
Diastolic blood pressure (mm Hg)	71.0 (69.9, 72.2)	71.6 (70.5, 72.8)	0.6 (−1.0, 2.2)	0.47
Pulse pressure (mm Hg)	51.9 (5.07, 53.2)	48.4 (47.2, 49.6)	−3.5 (−5.3, −1.8)	<0.001
Heart rate (beats/min)	71.4 (70.2, 72.6)	71.4 (70.2, 72.6)	0.0 (−1.7, 1.7)	0.99
Awake ambulatory blood pressure and heart rate				
Systolic blood pressure (mm Hg)	127.6 (124.9, 130.3)	124.6 (121.8, 127.3)	−3.1 (−6.4, 0.3)	0.08
Diastolic blood pressure (mm Hg)	74.7 (73.0, 76.5)	76.4 (74.6,78.2)	1.6 (−0.5, 3.7)	0.13
Pulse pressure (mm Hg)	52.9 (51.1, 54.8)	48.4 (46.5, 50.2)	−4.6 (−6.7, −2.5)	<0.001
Heart rate (beats/min)	74.7 (72.8, 76.6)	75.3 (73.4, 77.2)	0.6 (−1.5, 2.7)	0.56
Asleep ambulatory blood pressure and heart rate				
Systolic blood pressure (mm Hg)	113.6 (110.7, 116.5)	110.5 (107.6, 113.4)	−3.1 (−6.4, 0.2)	0.06
Diastolic blood pressure (mm Hg)	63.9 (61.8, 66.1)	63.1 (61.0,65.3)	−0.8 (−3.1, 1.5)	0.48
Pulse pressure (mm Hg)	49.7 (47.7, 51.7)	47.5 (45.5, 49.5)	−2.2 (−4.5, 0.1)	0.06
Heart rate (beats/min)	63.7 (61.7, 65.7)	62.7 (60.8, 64.7)	−1.0 (−3.5, 1.5)	0.44

[1] Values in this column are baseline-adjusted means (95% CIs).
[2] Values in this column are mean between-group differences (95% CIs).
[3] P value is for baseline-adjusted end-of-intervention differences analyzed with random-effects models.

often provides an important contribution to total energy intake. The bread intake achieved in our study (≈1600 kJ/d, 18% of total energy intake, 4 × 40–45-g slices/d) was moderate.

The observed effect of the current intervention on systolic blood pressure is consistent with the magnitude that might be predicted. In our population of men and women with blood pressures in the normal to mild hypertension range, a higher dietary fiber intake of 13 g/d would be predicted to result in lower systolic blood pressure by ≈1–1.5 mm Hg (12, 13). A predicted estimate of the effect of higher protein and lower carbohydrate intakes on systolic blood pressure is a little more difficult from results of previous controlled trials (6–10), because differences in protein and carbohydrate intake have usually been considerably higher, and the populations recruited and their background diets have varied. However, estimates from the INTERSALT Study (an international study of electrolyte excretion and blood pressure) that included >10,000 men and women with a mean systolic blood pressure of 119 mm Hg would predict that a higher protein intake of 14 g/d would result in a lower systolic blood pressure of ≈1 mm Hg (17). The population recruited to our study had a mean daytime ambulatory systolic blood pressure of ≈127 mm Hg. Effects on blood pressure may be a little greater in such a population. In addition, a lower carbohydrate intake of 20 g/d would be predicted to result in lower systolic blood pressure by ≈0.5 mm Hg (6–10). It is unlikely that the estimates for the effects of protein and carbohydrate would be additive, because of some degree of substitution of these nutrients in an isoenergetic diet.

Small differences in intakes of other nutrients, such as sodium, potassium, magnesium, and calcium, also have the potential to contribute to changes in blood pressure. The 2 breads in our study were matched for sodium content, and there were no differences between groups in sodium excretion or calcium intake. In addition, adjustment for changes in sodium or potassium excretion or in magnesium intake did not alter the observed differences. Although the between-group differences in potassium intakes were not significant, potassium and magnesium intakes were estimated to be higher by 274 (≈6 mmol/d) and 41 mg/d (≈2 mmol/d), respectively, for lupin compared with the control. Meta-analyses of trials of potassium (18) and magnesium (19) indicate that such small changes in intake are likely to have little effect on blood pressure.

The mechanisms behind the observed effects on blood pressure are uncertain. A previous study that used a salt-sensitive rat model of hypertension showed that lupin protein can attenuate the development of hypertension and improve endothelial function (20). Lupin protein has a relatively high content of arginine (21), which is a precursor for nitric oxide synthesis. The decrease in blood pressure could result from an improvement in vascular tone mediated by nitric oxide, a potent endothelium-derived relaxing factor (20). However, it is difficult to speculate on the mechanisms behind the observed differences in blood pressure, given that multiple factors in the diet—protein, carbohydrate, and fiber—were changed. A range of mechanisms may be involved. In addition, it seems likely that similar effects on blood pressure would be found with the use of other sources of protein and fiber to alter the composition of high-carbohydrate foods (12, 13, 22). The dietary source of protein may not be a major determinant of the effect on blood pressure (22). Nevertheless, lupin kernel flour is an ingredient that can effectively increase the protein and fiber contents of high-carbohydrate foods such as bread. In contrast, the source and form of carbohydrate in the diet may be an important determinant of the effect on blood pressure (22).

In the present study, regular consumption of the lupin bread, compared with the control bread, resulted in lower 24-h systolic blood pressure and pulse pressure. At a population level, the observed differences in systolic blood pressure would be associated with a 10% difference in the prevalence of hypertension, a 4% difference in the risk of coronary artery disease, and a 10% difference in the risk of stroke (23). The effects on pulse pressure may also be important because lower pulse pressure has been associated with lower rates of cardiovascular mortality (24–26).

In conclusion, we have shown that substituting 40% of the wheat flour in bread with lupin kernel flour resulted in higher

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protein and fiber intakes and lower systolic blood and pulse pressures in overweight men and women. These results suggest that a diet moderately higher in dietary protein and fiber can significantly reduce blood pressure. They also confirm the potential of lupin kernel flour as a novel food ingredient to bring about these outcomes. This approach may be a relatively simple and acceptable dietary measure for helping to reduce cardiovascular risk in overweight and obese persons.

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