Supplementation of Conventional Therapy With the Novel Grain Salba (*Salvia hispanica L.*) Improves Major and Emerging Cardiovascular Risk Factors in Type 2 Diabetes

Results of a randomized controlled trial

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OBJECTIVE — To determine whether addition of Salba (*Salvia hispanica L.*), a novel whole grain that is rich in fiber, α-linolenic acid (ALA), and minerals to conventional treatment is associated with improvement in major and emerging cardiovascular risk factors in individuals with type 2 diabetes.

RESEARCH DESIGN AND METHODS — Using a single-blind cross-over design, subjects were randomly assigned to receive either 37 ± 4 g/day of Salba or wheat bran for 12 weeks while maintaining their conventional diabetes therapies. Twenty well-controlled subjects with type 2 diabetes (11 men and 9 women, aged 64 ± 8 years, BMI 28 ± 4 kg/m^2, and A1C 6.8 ± 0.9%) completed the study. This study was set in the outpatient clinic of the Risk Factor Modification Center, St. Michael's Hospital, Toronto, Canada.

RESULTS — Compared with the control treatment, Salba reduced systolic blood pressure (SBP) by 6.3 ± 4 mmHg ($P < 0.001$), high-sensitivity C-reactive protein (hs-CRP) (mg/l) by 40 ± 1.6% ($P = 0.04$), and vonWillebrand factor (vWF) by 21 ± 0.3% ($P = 0.03$), with significant decreases in A1C and fibrinogen in relation to the Salba baseline but not with the control treatment. There were no changes in safety parameters including liver, kidney and hemostatic function, or body weight. Both plasma ALA and eicosapentaenoic polyunsaturated fatty acid levels were increased twofold ($P < 0.05$) while consuming Salba.

CONCLUSIONS — Long-term supplementation with Salba attenuated a major cardiovascular risk factor (SBP) and emerging factors (hs-CRP and vWF) safely beyond conventional therapy, while maintaining good glycemic and lipid control in people with well-controlled type 2 diabetes.

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Abbreviations: ALA, α-linolenic acid; CVD, cardiovascular disease; DBP, diastolic blood pressure; EPA, eicosapentaenoic acid; hs-CRP, high-sensitivity C-reactive protein; PUFA, polyunsaturated fatty acid; SBP, systolic blood pressure; vWF, vonWillebrand factor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Diabetes is a highly prevalent and heterogeneous condition, with cardiometabolic implications that can be improved by tight glycemic control. An aggressive reduction in major risk factors for cardiovascular disease (CVD), such as elevated blood pressure and dyslipidemia, as well as emerging risk factors, including proinflammatory and prothrombotic markers, is recommended. However, despite an armamentarium of medications and lifestyle therapy, these goals are often difficult to achieve, placing people with diabetes at increased CVD risk. New treatment modalities to complement existing interventions are therefore of great interest, including dietary interventions for primary prevention or as a possible therapeutic option that may confer benefits beyond currently recommended conventional therapies.

There is growing evidence that whole grains may play an important role in the prevention of chronic disease. Collective endorsement of whole grains by major health agencies around the world, including the Food and Drug Administration–approved health claim (1), is based on large epidemiological and prospective population studies that suggest a strong inverse relationship between increased consumption of whole-grain foods and reduced risk of diabetes and CVD (2). Populations that consume three or more servings per day may benefit from the cardioprotective benefits of whole grain. It is, however, unknown which of the constituents of whole grain are responsible for the benefit. Phytoprotective constituents, including dietary fiber, antioxidants, minerals, and vitamins, have been suggested, but the physiological mechanisms of the cardioprotective effects are still poorly understood. The main sources of whole grains in the diet are bread and breakfast cereals, which are relatively nutrient-depleted foods due to aggressive in-

dustrial processing. Introduction of new varieties of whole grain should be encouraged in order for the general public to best adhere to effective health strategies that promote whole-grain consumption. Furthermore, well-controlled intervention studies are required to provide information about the link of the specific nutrients from whole grains to cardiovascular health.

Salba is a new generation of whole grain produced by Salba Corporation, Buenos Aires, Argentina, and cultivated by selective breeding (AgriSalba, Ica, Peru). Salba is a white-color variety from the original herbaceous plant *Salvia hispanica L.*, which is >90% black grain and is known as a "running food" and used as both food and remedy by the ancient Aztecs. Salba is a pleasant-tasting grain that can easily be incorporated into a variety of baked products or just sprinkled onto yogurt, salad, soup, etc. With its rich nutrient composition, compared with most whole grains currently recommended, Salba represents the highest known whole-food source of dietary fiber and the n-3 polyunsaturated fatty acid (PUFA), α-linolenic acid (ALA), in nature. In addition, it is an exceptionally rich source of vegetable protein, calcium, magnesium, iron, and antioxidants (i.e., total antioxidant capacity is 70 per gram of Salba). As all these nutrients have been implicated in lowering CVD risks, and as they occur naturally in Salba, we hypothesized that simple addition of Salba to conventional treatment may reduce CVD risk factors when added to the diet of individuals with well-controlled type 2 diabetes.

RESEARCH DESIGN AND METHODS

— The study was approved by the St. Michael's Hospital Ethics Review Board. Eligible participants gave written informed consent. Eligibility criteria included the following: documented type 2 diabetes for at least 6 months' duration without clinically manifest complications, age 18–75 years, nonpregnant, metabolically stable (A1C 6.0–8.5% and fasting plasma glucose 6.4–8.5 mmol/l), and not taking insulin, dietary fiber supplements, ALA supplements, or fish oil or consuming cold-water fish more than three times per week. Subjects were instructed not to change their lifestyle and level of physical activity during the study. Assuming a 35% attrition rate and a two-tailed $\alpha = 0.05$ and $1\text{-}\beta = 80\%$ to detect a 0.8% difference in A1C with an SD of 0.8%, sample size calculations indicated that 27 participants needed to be enrolled to yield 19 participants for final analysis.

Treatments

The supplements were provided both in ground form and in specially formulated breads that were similar in appearance. Both the test and control intervention food were matched for energy and total dietary fiber. Salba was obtained from Salba Nutritional Solutions (Toronto, Canada), and the wheat bran control was from the American Association of Cereal Chemists (St. Paul, MN). The Salba and control breads were prepared in a bakery using a standardized recipe and methodology. The ground supplements (Salba and wheat bran control) were matched in appearance and were provided in opaque containers. Subjects were instructed to keep the bread and supplements in the refrigerator to minimize possible oxidation of the n-3 fats. Supplements were provided at a level of 15 g/1,000 kcal intake and were calculated according to subject's individual daily energy requirements, as estimated by the Harrison-Benedict equation multiplied by a "very light" activity factor of 1.3 and verified with the initial 3-day dietary record. Control supplements contained wheat bran, which is considered to be lipid neutral and has little effect on glucose tolerance (3).

Conventional therapy

Salba or control supplements were added to conventional therapy. Dietitians instructed the participants to consume a diet that followed the Canadian Diabetes Association nutrition recommendations (4). The targeted macronutrient profile of carbohydrate:protein:fat was ~55:15:30%, with an emphasis on low–glycemic index carbohydrate sources, 25–35 g total fiber, ≤10% sugars, and <10% saturated fatty acids. To monitor compliance, dietary data were analyzed at weeks 0 and 12 for energy and macro- and micronutrients. Three-day food records were analyzed by using Food Processor Nutrition Analysis software version 7.1 (ESHA Research, Salem, OR). Participants were also instructed to maintain their usual therapy (type and dose) of oral hypoglycemic, antihypertensive, or lipid-lowering medications. Salba or control supplements were added to this combination of a diet that followed Canadian Diabetes Association nutrition recommendations and usual therapy. By mimicking the macronutrient profile of the background Canadian Diabetes Association diets, the control supplements were designed to act as an extension of the diets.

Study design

The study used a randomized, placebo-controlled, single-blind, cross-over design with four distinct periods, in which subjects acted as their own control. Participants began with a run-in phase for at least 2 weeks to adjust to a healthy diet and to stabilize baseline parameters. Half of the participants were then randomized to either the Salba or control treatment for the first of two 12-week treatment arms. This period was followed by a 4- to 6-week washout phase to mitigate carryover effects. For the second treatment phase, participants were crossed-over to the alternate treatment. During each treatment phase, participants attended the clinic every 2 weeks to have anthropometric and clinical measurements taken, submit symptom diary and 3-day dietary records, receive new treatment foods, return unused bread and ground supplements, and have an interview with the dietitian and principal investigator. At weeks 0 and 12, fasting blood samples were collected.

Throughout the trial, an independent research assistant maintained the blinding of packages, labels, and diet randomization. Randomization was achieved using a computer-generated random-number table. Participants were excluded if during the course of the study there were any changes to their regular antihypertensive, lipid-lowering, or oral hypoglycemic medications; if they consumed <50% of the study supplements; or if they had a significant weight change over the course of the study, defined as >3 kg per 3 months.

Outcomes

There were three levels of outcome measures (efficacy, safety, and compliance) for which separate models were constructed. Efficacy measures included glycemic control (A1C, fasting plasma glucose, and fasting plasma insulin), blood pressure (office systolic blood pressure [SBP] and diastolic blood pressure [DBP]), lipids (total, LDL, and HDL cholesterol and triglycerides), and emerging risk factors for CVD (high-sensitivity C-reactive protein [hs-CRP], fibrinogen, von Willebrand factor [vWF], and factor VIII). Safety measures included markers of hepatic (aspartate aminotransferase

and alanine aminotransferase), renal (serum urea and creatinine), and hemostatic (prothrombin time, partial thromboplastin time, and international normalized ratio) function. Finally, compliance measures included body weight change, quantity of returned bread and ground Salba supplements at follow-up visits, lifestyle adherence (dietary profiles and body weight), and plasma fatty acids.

Measurements and analyses

Office blood pressure measurements were performed as described previously (5), with the cuff secured around the participant's nondominant arm. Samples for A1C and hepatic, renal, and hemostatic function were analyzed directly by St. Michael's Hospital Core laboratory. Plasma A1C was determined using ion-exchange high-performance liquid chromatography (Diamat HPLC; Bio-Rad Laboratories, Mississauga, Canada). Prothrombin time and partial thromboplastin time were determined using appropriate reagents and automatic clot timers. International normalized ratio was derived according to the following formula: (patient prothrombin time/mean normal prothrombin time)$^{\text{internal sensitivity index}}$. Serum alanine aminotransferase and aspartate aminotransferase were determined enzymatically. Serum creatinine and urea were determined by standard methods. Plasma glucose and insulin analyses were performed by the glucose oxidase and double-antibody radioimmunoassay methods, respectively. Plasma total lipids were analyzed by gas liquid chromatography (6).

Statistical methods

Statistical analyses were performed using NCSS 2000 (NCSS Statistical Software, Kaysville, UT). Comparisons of within- and between-treatment differences in compliance, efficacy, and safety outcomes were assessed using repeated-measures general linear model ANCOVA adjusted for sex, age, and sequence. Results were expressed as means ± SD, and significance was set at $P < 0.05$. The Newman-Keuls procedure was used post hoc to adjust for multiple comparisons. Between-treatment results were adjusted for baseline values. Percent changes for each variable are based on the calculation for each individual subject's percent change. All comparisons were adjusted for age, sex, weight, BMI, and use of cholesterol and hypotensive medications, aspirin, and oral hypoglycemic agents.

RESULTS — Forty-four subjects with type 2 diabetes were screened, and 27 eligible subjects were enrolled in the study. Exclusions during Salba treatment and control were equal. Reasons for dropouts during the protocol included medication changes ($n = 3$), refusal to continue ($n = 3$), and increased gastrointestinal side effects ($n = 2$). Final analysis included 20 patients with type 2 diabetes: 11 men and 9 women (means ± SD), aged 64 ± 8 years, BMI 28 ± 4 kg/m^2, and A1C 6.8 ± 0.9%. Medication treatment received concomitantly by the participants was single or combined oral hypoglycemic agents (10 subjects were on insulin secretagogues, 9 on metformin, and 1 on pioglitazone). Four subjects were on HMG-CoA (hydroxymethylglutaryl coenzyme A) reductase inhibitors (three on atorvastatin and one on simvastatin), three were on low-dose aspirin, and nine were on antihypertensive agents (four on ACE inhibitors, three on β-blockers, and two on calcium-channel blockers). Participants who had a change in their medications were excluded from the final analysis.

Compliance

Within- and between-treatment differences in markers of compliance were assessed for both Salba and control treatment. Neither the proportion of supplements (cumulative bread and ground supplement) consumed over the 12 weeks between Salba and the control treatment (82 ± 24 vs. 85 ± 6%) nor body weight change from week 0 to 12 (0.25 ± 0.42 vs. 0.18 ± 0.37 kg) was significantly different between the Salba and control treatment. Further proof of compliance is supported by the results of the plasma total fatty acid analysis assessed at the end of each treatment. After 12 weeks of consuming Salba, the participants had approximately double the plasma level of ALA (2.5 ± 0.3 vs. 1.2 ± 0.1%; $P < 0.05$) and eicosapentaenoic acid (EPA) (0.18 ± 0.03 vs. 0.09 ± 0.02%; $P = 0.006$), when compared with the control treatment.

Diet

Analysis of the 3-day dietary records showed that participants maintained the diets they were instructed to follow. The addition of Salba to a healthy diet changed the macronutrient intakes between the Salba and control treatment with respect to the percent of calories from carbohydrate and fat. In the Salba group, the dietary profile was 45:21:34 (% calories from carbohydrate:protein:fat), whereas in the control diet group it was 54:19:27. Both total fat and PUFAs were significantly higher in the Salba treatment than control groups ($P < 0.001$) at the expense of carbohydrate, which was significantly lower in the Salba than control group ($P < 0.001$). The monounsaturated fatty acid content of the Salba phase was higher at $P = 0.04$. As expected, the n-3 PUFA intake was also significantly higher and reached 7.4 ± 4.3 g/day compared with 1.1 ± 0.8 g/day on the control treatment ($P < 0.001$). The mineral intakes were not significantly different except for magnesium, which was significantly higher ($P = 0.03$) with Salba (612 ± 149 mg) due to the high content of magnesium in Salba compared with the control diet (424 ± 167 mg).

Efficacy parameters

Major CVD risk factors: blood pressure, A1C, fasting glucose, insulin, and serum lipids. Compared with baseline, SBP dropped on the Salba treatment by 6.3 ± 4.2 mmHg ($P < 0.001$) to an average of 123 ± 16 mmHg. DBP dropped by 3 ± 1.3 mmHg on the Salba treatment but did not reach statistical significance (Table 1). On the control treatment, the SBP increased by 7 ± 1.17 mmHg and the DBP increased by 3.14 ± 1.12 mmHg; as a result, SBP achieved statistical significance at $P < 0.05$, with a 20 ± 1% reduction and a nonsignificant 7% reduction for DBP between the Salba and the control treatment. Mean measures of glycemic control at the end of the Salba phase (A1C, fasting blood glucose, and fasting blood insulin) were not statistically different when compared with the end of the control phase (Table 1). No significant difference was observed comparing change from baseline in any parameter on the control phase. Fasting plasma blood glucose and insulin levels were ~3% lower after treatment with Salba, but this did not reach significance. A1C was significantly reduced from baseline during the Salba treatment ($P = 0.02$) but not when compared with the control treatment.

Blood lipids were not different between the Salba and control treatments in any measured parameter (triglycerides and LDL, HDL, and total cholesterol) (Table 1). Baseline lipids were within the targets set by the 2006 Clinical Practice Guidelines (7), with the exception of LDL cholesterol, which was marginally ele-

Table 1—*Changes in major and emerging CVD risk factors during and between the Salba and control treatments* (n = 20)

	Salba			Control			Between treatment	
	Week 0	Week 12	Change (%)	Week 0	Week 12	Change (%)	Change (%)	*P* value
Major CVD risk factors								
A1C (%)	6.9 ± 0.8	6.7 ± 0.9	−2.9 ± 8.4*	6.7 ± 0.8	6.7 ± 0.9	−0.2 ± 6.7	−2.6 ± 12.4	0.37
Fasting blood glucose (mmol/l)	7.4 ± 2.1	7.2 ± 1.9	−0.7 ± 21.7	7.1 ± 1.5	7.6 ± 1.9	4.1 ± 11.6	−7.3 ± 25.5	0.12
Fasting blood insulin (pmol/l)	74.4 ± 28.8	86.1 ± 39.1	17.5 ± 34.7	82.2 ± 35.6	85.1 ± 44.4	3.5 ± 25.2	14.0 ± 43.2	0.24
Total cholesterol (mmol/l)	5.1 ± 1.2	4.9 ± 1.2	−2.3 ± 1.2	5.0 ± 1.2	5.0 ± 1.2	0.5 ± 1.2	−2.8 ± 0.6	0.47
LDL cholesterol (mmol/l)	3.0 ± 1.0	3.0 ± 1.0	−1.2 ± 1.0	2.9 ± 0.9	3.0 ± 1.0	2.0 ± 1.0	−3.2 ± 0.4	0.56
HDL cholesterol (mmol/l)	1.3 ± 0.2	1.2 ± 0.3	−5.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.2	−1.5 ± 0.3	−3.7 ± 0.1	0.26
Triglycerides (mmol/l)	1.6 ± 0.8	1.6 ± 0.8	−1.7 ± 0.8	1.7 ± 0.9	1.7 ± 0.8	−2.1 ± 0.8	0.4 ± 0.4	0.95
SBP (mmHg)	129 ± 17	123 ± 16	−9.5 ± 1*	122 ± 15	129 ± 17	10 ± 2*	−20 ± 1	>0.01
DBP (mmHg)	81 ± 9	78 ± 8	−3 ± 1.3	76 ± 9	79 ± 7	9.2 ± 1.3*	−7 ± 1	>0.05
Emerging CVD risk factors								
hs-CRP (mg/l)	3.1 ± 2.4	2.9 ± 2.3	−7.0 ± 2.3	2.6 ± 2.1	3.4 ± 2.3	32.9 ± 2.2*	−39.9 ± 1.6	0.04
Fibrinogen (g/l)	3.52 ± 0.76	3.37 ± 0.61	−5.32 ± 0.68*	3.24 ± 0.69	3.35 ± 0.71	3.57 ± 0.69	−8.89 ± 0.52	0.1
vWF	1.14 ± 0.38	1.038 ± 0.45	−9.12 ± 0.41	1.158 ± 0.66	1.299 ± 0.61	12.13 ± 0.63	−21.25 ± 0.34	0.03
Factor VIII	1.04 ± 0.36	0.95 ± 0.36	−8.68 ± 0.36	0.85 ± 0.39	0.97 ± 0.47	14.3 ± 0.43	−22.98 ± 0.29	0.06

Data are means ± SD and represent measures of efficacy. The safety and compliance data are described in the text. *P* values refer to between-treatment differences using repeated-measures general linear model ANOVA adjusted for age, sex, weight, antihyperglycemic agents (A1C, fasting blood glucose, and fasting blood insulin); for age, sex, weight, BMI, lipid-lowering medications (total, LDL, and HDL cholesterol; triglycerides; and hs-CRP); for age, sex, weight, and aspirin use (fibrinogen, vWF, and factor VIII); and for age, sex, weight, and blood pressure medications (SBP and DBP). Percent changes for each variable are based on the calculation for each individual subject's percent change. *$P < 0.05$.

vated (primary target LDL cholesterol ≤2.0 mmol/l).

Emerging CVD risk factors: coagulation and inflammatory markers. On the Salba treatment, fibrinogen levels significantly decreased over the 12 weeks, from 3.52 ± 0.76 to 3.37 ± 0.61 g/l (P = 0.03), but was not significantly different from the control treatment (Table 1). At the end of the Salba phase, vWF was significantly lower than in the control treatment (P = 0.03), while factor VIII was reduced by 23%, although this did not reach statistical significance ($P < 0.06$) when compared with the control treatment. Low-grade body inflammation measured by hs-CRP was not significantly different from baseline in the Salba phase (−7%, P = 0.24) but increased significantly (33%) during the control phase (P = 0.01) and was significantly higher at the end of the control phase compared with the end of the Salba phase (40%, P = 0.04).

Safety

Mean international normalized ratio, prothrombin time, and partial thromboplastin time did not change over the 12 weeks of the study period in either the Salba or the control treatments (data not shown). Mean values of blood urea nitrogen and creatinine, both measurements of kidney function, were not significantly different. Aspartate aminotransferase and alanine aminotransferase, measures of liver function, also did not change. Both baseline and postintervention values were within normal ranges as set by St. Michael's Hospital Core Laboratory.

CONCLUSIONS

This study demonstrated that a 12-week dietary supplementation with the novel whole grain Salba (*Salvia hispanica L.*) was associated with attenuated SBP and the emerging risk factors (hs-CRP and vWF) in people with type 2 diabetes controlled on diet and oral hypoglycemic agents.

An increasing body of evidence from epidemiological observational studies suggests a strong inverse relationship between consumption of whole grain and the risk of diabetes and CVD (8). Many components of whole grains, including complex carbohydrates, vegetable protein, n-3 PUFAs, dietary fiber, minerals, antioxidants, and their combined effects could be responsible for this reduction. However, controlled interventional studies supporting health claims for whole grain are scarce. Therefore, our study was

designed to determine whether the novel whole grain Salba could reduce CVD risk factors when added to usual care of individuals with well-controlled type 2 diabetes. To our knowledge, this is one of the first long-term randomized controlled trials to demonstrate a simultaneous reduction of major and emerging CVD risk factors in an intervention using whole grain in well-controlled diabetes.

All subjects in the study consumed >50% of supplements and were compliant with the treatment diet. Twelve-week dietary supplementation with 37 ± 4 g/day of Salba (7 g/day ALA) resulted in a twofold elevation in plasma phospholipid EPA, with no change of docosahexaenoic acid, compared with the control treatment. Similar changes were observed in another study (9) in which dietary supplementation of 9.5 g ALA per day during 3 months resulted in an 87% increase in plasma total lipid EPA compared with the control treatment. A parallel 1.7-g/day EPA plus docosahexaenoic acid supplementation elevated plasma phospholipid EPA similarly, by 79% (9).

This study demonstrated the safety of adding 37 g of Salba to a traditional healthy diet and conventional therapy. Although Salba has been used for many years in the diets of Aztecs in Mexico with no apparent side effects, there was a concern that a high amount of n-3 PUFAs could alter the eicosanoids produced, leading to an adverse effect on clotting factors. High doses of n-3 PUFAs have been implicated in altered bleeding and clotting time (10). However, coagulation, liver enzymes, and kidney function were not significantly affected by the addition of Salba in our study.

Major cardiovascular risk factors

Despite the fact that our acute pilot study demonstrated that bread made with Salba reduced postprandial glucose and insulinemia (11), there were no long-term beneficial effects of Salba treatment on fasting blood glucose and insulin. Although a significant change was seen across Salba treatment in A1C, this was not significant when compared with control treatment, presumably due to the already optimal baseline glycemic control (A1C 6.8 ± 0.9%) achieved by subjects' underlying diabetes therapy. Many factors in Salba may be responsible for this moderate glycemic-lowering effect. Salba contains 36% of its weight as fiber, of which only 4 g are soluble fiber but of a very high viscosity. The viscous mucilage formed when fiber from Salba is exposed to water and human digesta might be one of the main factors potentially thought to affect glycemic control. Factors in Salba that may act against good glycemic control include high content of n-3 PUFAs, which in some studies has been shown to increase A1C and fasting and postprandial glycemia in type 2 diabetes (12).

Dietary n-3 PUFAs have been associated with the reduction of serum triglycerides but at the expense of increasing LDL cholesterol levels. In the Lyon Diet Heart Study (13), the addition of n-3 PUFAs to a high-carbohydrate low-fat Mediterranean diet did not affect triglycerides or LDL, HDL, or total cholesterol, yet it still produced a 65% reduction in coronary heart disease mortality, indicating that changes in traditional risk factors such as blood lipids are not the sole cause of coronary heart disease. Our study provides evidence that 37 g of Salba (7 g ALA) has no detrimental effect on blood lipid profile in a group of individuals with type 2 diabetes, who are typically two to four times more susceptible to heart disease than the nondiabetic population.

Diet has long been implicated in reducing blood pressure. In addition, the micronutrients potassium (K), calcium (Ca), and magnesium (Mg) have also demonstrated blood pressure–lowering effects both individually and in combination (14). With the exception of higher quantity of n-3 PUFAs and magnesium in Salba, the treatments were not significantly different in known factors affecting blood pressure such as protein, fiber, sodium, potassium, and calcium. Although nine subjects were receiving antihypertensive medications, both SBP and DBP dropped on the Salba treatment, although DBP did not achieve statistical significance, indicating that the drug therapy did not obscure the response of blood pressure to n-3 treatment (15). It can be speculated that the mechanism of the reduction seen in SBP, and possibly DBP, could be due a conversion of Salba's ALA to EPA, leading to the production of less vasoconstrictive prostaglandins through modification of the eicosanoid pathway. Most intervention studies show the benefits of fish oil and EPA on blood pressure (16).

Emerging cardiovascular risk factors

Inflammation plays a major role in CVD, and measurement of inflammatory markers such as hs-CRP may be beneficial for risk stratification (17). Many of the previous prospective studies evaluating the effect of either fish oil or ALA on hs-CRP did not find significant changes in hs-CRP levels with n-3 PUFA consumption (18). However, in several recent randomized controlled trials (19,20) conducted in hypercholesterolemic subjects, consumption of ALA diets significantly decreased serum levels of CRP. Consistent with these findings, our results demonstrate that consumption of Salba, a grain naturally high in ALA, resulted in a significant decrease of hs-CRP, and the changes in serum ALA and EPA were inversely associated with changes in CRP. More recently, it has been suggested that increased levels of EPA alone may be cardioprotective. This is supported by the results of our study and a recent study that demonstrated that supplementation of purified EPA over 3 months to individuals with the metabolic syndrome resulted in reductions in small dense LDL particles and CRP levels (21).

Fibrinogen is negatively associated with the relative risk for CVD and cardiovascular risk factors (22). Although fibrinogen level was not different from the control treatment, it significantly decreased from baseline on the Salba by 0.15 g/l. A systematic review by Wendland et al. (23) indicated that ALA significantly affects fibrinogen concentrations, decreasing them by 0.17 mmol/l. This reduction would be expected to lead to a 6% decrease in coronary heart disease.

Although vWF is only weakly associated with the risk of CVD in the general population, it is more significant in high-risk populations such as people with diabetes (24). Salba treatment decreased the plasma level of this risk factor by 21%, but whether it could be attributed to n-3 PUFA or other components of the grain is unclear. There was a trend toward reduction of factor VIII (by 23%) in our study. Similarly, previous studies demonstrated either no change (25) or decrease (26) in activity of this factor with ALA or fish oil supplementation.

Implications of preliminary results

A simple whole-grain dietary intervention with Salba may potentially play an important role in the primary prevention of type 2 diabetes by increasing adherence to the recommended three servings per day, as well as being a therapeutic option that could be effective beyond currently recommended conventional therapies in improving major and emerging cardiovascular risk factors in type 2 diabetes. The

amount of Salba consumed daily on this study was equivalent to the two portions of whole grain, as recommended by the Food and Drug Administration.

Limitations

The study was designed to determine the safety and efficacy of consuming the novel whole grain Salba when added to the diet of individuals with type 2 diabetes while on conventional diabetes therapies. In such a design, no specific functional component from Salba treatment can clearly be implicated in any effect seen, but it is comparable with the cardioprotective effect seen in other whole grains. In addition to the numerous functional components in Salba, the supplementation of Salba produced changes in the dietary macronutrient profile between the two treatments. Therefore, the changes in risk factors observed could possibly be explained by the change in the carbohydrate and fat composition of the different diets (27), and future studies should compare Salba with control supplements that are matched for macronutrient composition. Furthermore, although subjects were instructed not to change their level of physical activity, the physical activity level during the study was not quantified, which might bias the results. Finally, our main objective was to assess the effect of Salba on A1C in subjects with type 2 diabetes, and the power of the study, limited to 20 participants, may not be sufficient for other than A1C parameters. Although the participants who completed the study were stable and well controlled, they may not represent typical candidates for adjunctive therapy. Both of these cavities render the conclusion that larger studies need to be conducted with the need for intent-to-treat analysis instead of per-protocol analysis.

In conclusion, the novel whole grain Salba, which can be simply added to conventional treatment for type 2 diabetes, improves major and emerging CVD risk factors while maintaining good glycemic and lipid control in well-controlled type 2 diabetes. The systolic blood pressure was reduced by 6.3 ± 4 mmHg, despite the fact that 9 of 20 participants were taking antihypertensive medications, which were not changed throughout the course of the study. As Salba contains a high amount of n-3 PUFAs, high dietary fiber, vegetable protein, and magnesium, the combination may have resulted in the pronounced blood pressure–lowering effects seen on the Salba treatment. Emerging CVD risk factors (hs-CRP and vWF) were attenuated beyond usual therapy (three subjects taking low-dose aspirin) on the Salba diet. This effect could be attributed to a high content of ALA in the Salba treatment and possibly the subsequent increase in EPA blood levels. No adverse effects of the conventional diet supplemented with the addition of Salba were documented, and liver function, kidney function, coagulation, and bleeding time were normal. In addition, there were no adverse effects on fasting plasma glucose, A1C, or LDL cholesterol, unlike previous studies with high doses of n-3 PUFAs in individuals with diabetes.

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References

1. U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition: A food labeling guide [article online], 1994. Available from http://www.cfsan.fda.gov/~dms/flg-6c.html. Accessed 14 February 2007
2. Richardson DP: Wholegrain health claims in Europe. *Proc Nutr Soc* 62:161–169, 2003
3. Jenkins DJ, Wolever TM, Leeds AR, Gassull MA, Haisman P, Dilawari J, Goff DV, Metz GL, Alberti KG: Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity. *Br Med J* 1:1392–1394, 1978
4. Wolever TMS, Barbeau MC, Charron S, Harrington K, Leung S, Madrick B, Taillefer T, Stero C: Guidelines for the nutritional management of diabetes mellitus in the new millennium: a position by the Canadian Diabetes Association. *CJDC* 23: 56–69, 2000
5. Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: The sixth report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Arch Intern Med* 157:2413–2446, 1997
6. Bazinet RP, McMillan EG, Cunnane SC: Dietary alpha-linolenic acid increases the n-3 PUFA content of sow's milk and the tissues of the suckling piglet. *Lipids* 38: 1045–1049, 2003
7. Canadian Diabetes Association: Clinical practice guidelines expert committee: dyslipidemia in adults with diabetes. *CJDC* 30:230–240, 2006
8. Seal CJ: Whole-grains and CVD risk. *Proc Nutr Soc* 65:24–34, 2006
9. Finnegan YE, Minihane AM, Leigh-Firbank EC, Kew S, Meijer GW, Muggli R, Calder PC, Williams CM: Plant- and marine-derived n-3 polyunsaturated fatty acids have differential effects on fasting and postprandial blood lipid concentrations and on the susceptibility of LDL to oxidative modification in moderately hyperlipidemic subjects. *Am J Clin Nutr* 77:783–795, 2003
10. Harper CR, Jacobson TA: The fats of life: the role of omega-3 fatty acids in the prevention of coronary heart disease. *Arch Intern Med* 161:2185–2192, 2001
11. Bazinet RP, Sievenpiper JL, Stavro PM, Cunnane SC, Vuksan V: *Salvia hispanica L.*, a rich source of alpha-linolenic acid, prolongs postprandial glycemia. *FASEB* 15:A992, 2001
12. Mori TA, Burke V, Puddey IB, Watts GF, O'Neal DN, Best JD, Beilin LJ: Purified eicosapentaenoic and docosahexaenoic acids have differential effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin in mildly hyperlipidemic men. *Am J Clin Nutr* 71:1085–1094, 2000
13. de Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, Mamelle N: Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. *Circulation* 99: 779–785, 1999
14. Suter PM, Sierro C, Vetter W: Nutritional factors in the control of blood pressure and hypertension. *Nutr Clin Care* 5:9–19, 2002
15. Bao DQ, Mori TA, Burke V, Puddey IB, Beilin LJ: Effects of dietary fish and weight reduction on ambulatory blood pressure in overweight hypertensives. *Hypertension* 32:710–717, 1998
16. Morris MC, Sacks F, Rosner B: Does fish oil lower blood pressure? A meta-analysis of controlled trials. *Circulation* 88:523–533, 1993
17. Ridker PM: High-sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. *Circulation* 103: 1813–1818, 2001
18. Balk EM, Lichtenstein AH, Chung M, Kupelnick B, Chew P, Lau J: Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review. *Atherosclerosis* 189:19–30, 2006
19. Zhao G, Etherton TD, Martin KR, West SG, Gillies PJ, Kris-Etherton PM: Dietary alpha-linolenic acid reduces inflammatory and lipid cardiovascular risk factors

in hypercholesterolemic men and women. *J Nutr* 134:2991–2997, 2004
20. Bemelmans WJ, Lefrandt JD, Feskens EJ, van Haelst PL, Broer J, Meyboom-de Jong B, May JF, Tervaert JW, Smit AJ: Increased alpha-linolenic acid intake lowers C-reactive protein, but has no effect on markers of atherosclerosis. *Eur J Clin Nutr* 58: 1083–1089, 2004
21. Satoh N, Shimatsu A, Kotani K, Sakane N, Yamada K, Suganami T, Kuzuya H, Ogawa Y: Purified eicosapentaenoic acid reduces small dense LDL, remnant lipoprotein particles, and C-reactive protein in metabolic syndrome. *Diabetes Care* 30:144–146, 2007
22. Rosenson RS, Koenig W: Utility of inflammatory markers in the management of coronary artery disease. *Am J Cardiol* 92: 10i–8i, 2003
23. Wendland E, Farmer A, Glasziou P, Neil A: Effect of alpha linolenic acid on cardiovascular risk markers: a systematic review. *Heart* 92:166–169, 2006
24. Vischer UM: von Willebrand factor, endothelial dysfunction, and cardiovascular disease. *J Thromb Haemost* 4:1186–1193, 2006
25. Allman-Farinelli MA, Hall D, Kingham K, Pang D, Petocz P, Favaloro EJ: Comparison of the effects of two low fat diets with different alpha-linolenic: linoleic acid ratios on coagulation and fibrinolysis. *Atherosclerosis* 142:159–168, 1999
26. Lox CD: The effects of dietary marine fish oils (omega-3 fatty acids) on coagulation profiles in men. *Gen Pharmacol* 21:241–246, 1990
27. Garg A, Bantle JP, Henry RR, Coulston AM, Griver KA, Raatz SK, Brinkley L, Chen YD, Grundy SM, Huet BA, Reaven GM: Effects of varying carbohydrate content of diet in patients with non-insulin-dependent diabetes. *JAMA* 271:1421–1428, 1994