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Effects of stabilized rice bran, its soluble and fiber fractions on blood glucose levels and serum lipid parameters in humans with diabetes mellitus Types I and II

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Abstract

Stabilized rice bran (SRB), a source of complex carbohydrates, tocols, γ-oryzanols, and polyphenols, was treated with carbohydrases and heat to yield two fractions, rice bran water solubles (RBWS), and rice bran fiber concentrates (RBFC). Stabilized rice bran and its fractions were fed for 60 days to insulin-dependent and noninsulin-dependent diabetes mellitus (IDDM = Type I and NIDDM = Type II) subjects to determine possible effects on serum hemoglobin, carbohydrate and lipid parameters. The Type I subjects (n = 22, 26, and 20) fed Stabilized rice bran, rice bran water solubles, and rice bran fiber concentrates plus AHA Step-1 diet reduced glycosylated hemoglobin 1%, 11%, and 10%, respectively. The fasting serum glucose levels were also reduced significantly ($P < 0.01$) with stabilized rice bran (9%), rice bran water solubles (29%), and rice bran fiber concentrates (19%).

The Type II subjects (n = 31, and 26) fed rice bran water solubles and rice bran fiber concentrates plus AHA Step-1 diet had decreased levels of glycosylated hemoglobin (15% and 11%) and fasting glucose (33% and 22%; $P < 0.001$), respectively. Serum insulin levels were increased (4%) with rice bran water solubles in both types of diabetes. The reduction of glycosylated hemoglobin and a slight increase in insulin levels indicate that consumption of rice bran water solubles can control blood glucose levels in human diabetes. Serum total cholesterol, LDL-cholesterol, apolipoprotein B, and triglycerides levels were reduced with rice bran fiber concentrates in the Type I (10, 16, 10, 7%) and Type II groups (12, 15, 10, 8%), respectively. These results indicate that rice bran water solubles significantly reduces hyperglycemia ($P < 0.01$), whereas rice bran fiber concentrates reduces hyperlipidemia ($P < 0.05$) in both types of diabetes. Therefore, these natural products can be used as nutritional supplements for the control of both types of diabetes mellitus in humans.

Keywords: stabilized rice bran (A); rice bran water solubles (B); rice bran fiber concentrates (C); diabetes mellitus Type I (IDDM) and Type II (NIDDM); serum glycosylated hemoglobin; glucose; insulin; lipid parameters

1. Introduction

Stabilized rice bran (a natural by-product of rice milling) fed to chickens for 4 weeks resulted in decreases in serum glucose, total cholesterol and LDL-cholesterol levels by 18, 22, and 34%, respectively [1]. The next step would be to determine if similar effects of stabilized rice bran can also be observed in 2 types of human diabetes and thus providing a safe and effective dietary supplement to control serum glucose level without any side-effects. The two most prevalent forms of diabetes are Type I (insulin-dependent diabetes mellitus [IDDM]), and Type II (noninsulin-dependent diabetes mellitus [NIDDM]). The Type I is referred to as juvenile-onset diabetes (between the ages 10 and 20) and always requires insulin for control [2]. The Type II is the most prevalent, and occurs mainly in people over 40 [2].

Hyperglycemia, hyperglycouria, hyperlipidemia and glycation/glycoxidation as well as the resultant atherosclerotic plague are among the characteristics of Types I and II diabetes [3–8]. Other than insulin injection, dietary controls such as decreased intake of refined carbohydrate and increased intake of certain dietary fibers have been demonstrated to lower postprandial glycouria and hyperglycemia by enhancing the tissue sensitivity to in-

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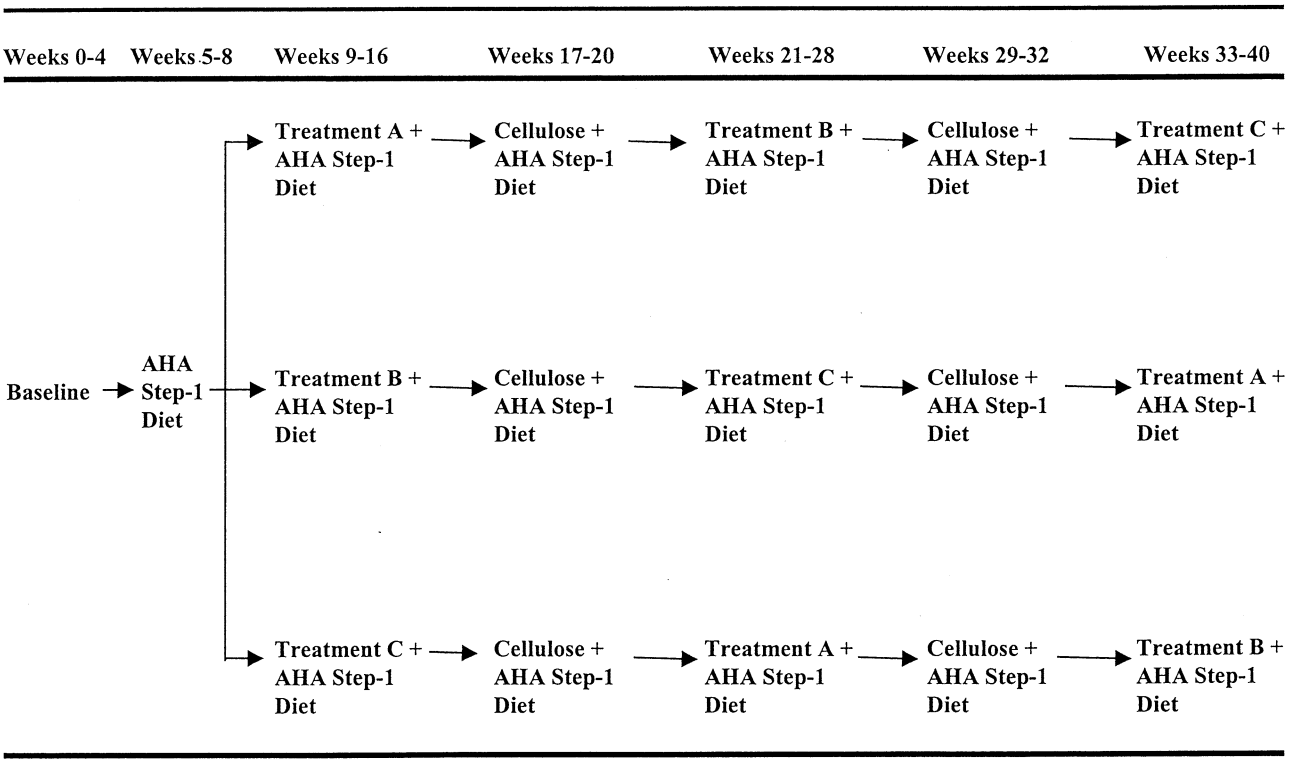

Fig. 1. Complete study design of the effects of stabilized rice bran (A), its subfractions-rice bran water solubles (B) and rice bran fiber concentrates (C) for both Types (I and II) of diabetes in human subjects.

sulin and slowing the absorption rate [9–14]. However, these measures still cannot control the fluctuation of blood sugar levels. Stabilized rice bran or its subfractions may satisfy this need. These considerations led to the study of hypoglycemic and hypocholesterolemic effect of stabilized rice bran or its subfractions in human subjects with diabetes.

Commercial rice bran, a milling by-product, contains 18–22% oil and is largely used as an animal feed stock. It is underutilized resource of value-added products. An endogenous enzyme (lipase) activates during milling, resulting in rapid deterioration of the oil, rendering it unsuitable for consumption [15,16]. Lipase enzyme is inactivated by heating the rice bran at 130–140°C for 3–90 sec, yielding stabilized rice bran [1,15–17]. Stabilized rice bran contains complex carbohydrates (starch and fibers) and microcomponents and it is a good source of a stable food grade oil [17]. The stabilized rice bran fractionates into rice bran water solubles (RBWS) and rice bran fiber concentrates (RBFC) when treated with carbohydrate-cleaving enzymes (dextranase, or α-amylase or maltase) in water and heated at 70–90°C [18]. The rice bran water solubles contain simple carbohydrates as dextrins, and rice bran fiber concentrates contain high levels of dietary fibers (β-glucan, pectin, and gums). Both fractions contain microcomponents found in stabilized rice bran [18]. Stabilized rice bran is Generally Regarded As Safe (GRAS) by the FDA and being tested in hypercholesterolemic human subjects for lowering their total cholesterol and LDL- cholesterol levels [19–23].

The present study investigates the effects of stabilized rice bran (SRB), rice bran water solubles (RBWS), and rice bran fiber concentrates (RBFC) in conjunction with American Heart Assosiation (AHA) Step-1 diet on serum glucose, insulin, glucagon levels, and other lipid parameters (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, apolipoprotein A-1, apolipoprotein B,

Table 1
Composition of stabilized rice bran, rice bran water solubles, and rice bran fiber concentrates

Ingredients	Stabilized rice bran (%)	Rice bran water solubles (%)	Rice bran fiber concentrates (%)
Carbohydrates	51.0*	57.5**	52.5**
Protein	14.5	7.5	20.5
Dietary Fiber	29.0	6.0	42.0
Fat	20.5	26.5	13.5
Tocols (>90% Tocotrienols)	350 ppm	270 ppm	30 ppm
γ-Oryzanol	3000 ppm	2600 ppm	2400 ppm
Microcomponents (γ-oryzanol, tocols, polyphenols, terpenes)	<1.1%	<0.77%	<0.92%

* Complex carbohydrates (starch).

** Starch is converted to dextrins after enzyme treatment of stabilized rice bran.

Table 2
Number of subjects of Type I and Type II diabetics completed studies of stabilized rice bran and its subfractions

Treatment product	Number of subjects of each treatment					Total subjects
	ABC	ABC + BC	A	B	C	
Type I						
Stabilized rice bran (A), $n = 22$	11		11			22
Rice bran water solubles (B), $n = 26$	11→	11 + 1		14		15
Rice bran fiber concentrates (C), $n = 20$		12			8	8
Total subjects on various treatments, $n = 68$						45
Type II						
Stabilized rice bran (A), $n = 23$	18		5			23
Rice bran water solubles (B), $n = 31$	18→	18 + 3		10		13
Rice bran fiber concentrates (C), $n = 26$		21			5	5
Total subjects on various treatments, $n = 80$						41

Two sachets (10 gm of each) of product A, B, or C were administered per day for 8-wk.

Lp(*a*), thromboxane B_2, and platelet factor 4) in human subjects with both types (I and II) of diabetes.

2. Materials and methods

2.1. Sources of chemicals and diagnostic kits

Sources of chemicals, substrates, and diagnostic kits have been identified previously [24–26]. Chemicals and solvents were of analytical grade. Glycosylated hemoglobin was estimated by using Glyco Hemoglobin Reagent Set, Horizon Diagnostics, Ann Arbor, Michigan, USA. Glucometers were supplied by Diabetes Resource Center, Inc., 175 5th Street S. W. Suite 300, P. O. Box 645, Winter Haven, FL. Sigma Diagnostic Kits were used to estimate serum glucose (# 315, 505 nm), total cholesterol (# 352, 500 nm), LDL-cholesterol (# 352, 500 nm), HDL-cholesterol (#352, 500 nm), triglycerides (# 336, 500 nm), apolipoprotein A-I

Table 3
Impact of stabilized rice bran and its subfractions on characteristics of the study population of Type I and II diabetics human subjects[1]

Characteristics	Stabilized rice bran (A)		Rice bran water solubles (B)		Rice bran fiber concentrates (C)	
	Before baseline	After (A)	Before cellulose + baseline	After (B)	Before cellulose + baseline	After (C)
Subjects						
Type I males	13	13	18	18	13	13
Type I females	9	9	8	8	7	7
Type II males	16	16	22	22	20	20
Type II females	7	7	9	9	6	6
Body weight (kg)						
Type I	69.86 ± 9.56	71.18 ± 10.21	69.54 ± 9.96	70.15 ± 9.89	70.30 ± 10.12	70.40 ± 9.78
Type II	68.48 ± 9.74	67.39 ± 19.48	68.87 ± 9.16	68.00 ± 9.53	69.23 ± 8.79	68.42 ± 9.50
Height (cm)						
Type I	162.82 ± 9.87	162.84 ± 9.95	165.81 ± 9.67	165.84 ± 8.92	161.11 ± 9.34	161.21 ± 8.76
Type II	162.17 ± 7.84	162.17 ± 7.95	163.06 ± 8.14	162.16 ± 8.26	163.19 ± 7.90	163.23 ± 7.98
Body mass index (kg/m^2)						
Type I	26.35 ± 4.31	26.84 ± 4.27	25.54 ± 3.89	25.51 ± 3.96	27.08 ± 4.37	27.09 ± 4.28
Type II	26.04 ± 3.96	25.62 ± 4.11	26.59 ± 3.96	25.86 ± 3.75	25.99 ± 4.21	25.68 ± 4.34
Energy (kj/d)						
Type I	6302 ± 1648	6187 ± 1461	6272 ± 1291	6245 ± 1210	6267 ± 1210	6239 ± 1146
Type II	6462 ± 1057	6442 ± 1059	6783 ± 1115	6769 ± 1176	6574 ± 1096	6564 ± 1068
Protein (g/d)						
Type I	70.01 ± 14.77	68.77 ± 15.22	69.92 ± 14.88	69.38 ± 19.96	69.05 ± 15.32	69.07 ± 15.70
Type II	68.00 ± 11.60	67.65 ± 11.24	68.55 ± 11.78	68.39 ± 12.55	69.04 ± 11.58	68.92 ± 11.15
Fat (g/d)						
Type I	69.64 ± 9.35	68.32 ± 9.68	70.50 ± 9.78	68.96 ± 9.59	69.60 ± 10.69	68.55 ± 9.04
Type II	64.74 ± 14.60	63.30 ± 14.24	66.48 ± 13.43	65.81 ± 13.16	61.88 ± 13.24	61.38 ± 13.02
Carbohydrate (g/d)						
Type I	194.91 ± 15.14	193.06 ± 15.71	186.88 ± 17.76	186.31 ± 17.64	189.55 ± 18.97	188.85 ± 19.33
Type II	169.13 ± 23.68	167.52 ± 25.07	176.90 ± 25.42	175.74 ± 26.39	170.46 ± 22.41	169.31 ± 23.95

[1] Data expressed as mean ± SD; Type I: product A ($n = 22$), product B ($n = 26$), product C ($n = 20$), Type II: product A ($n = 23$), product B ($n = 31$), and product C ($n = 26$).

Table 4
Effects of stabilized rice bran and its subfractions on glycemic parameters in Type I diabetes (Insulin-Dependent Diabetes Mellitus, IDDM) human subjects[1]

Glycemic parameters[2]	Stabilized rice bran (A; $n = 22$)		Rice bran water solubles (B; $n = 26$)		Rice bran fiber concentrates (C; $n = 20$)	
	Before baseline	After (A)	Before cellulose + baseline	After (B)	Before cellulose + baseline	After (C)
Phlebotomy data (serum)						
Glycosylated hemoglobin (%)	$10.89 \pm 2.13^{a,*}$ (100)[3]	10.82 ± 1.93^{a} (99)	11.25 ± 2.58^{a} (100)	10.06 ± 2.13^{a} (89)	11.32 ± 2.28^{a} (100)	10.23 ± 1.72^{a} (90)
Fasting glucose (*mmol/L*)	9.59 ± 1.23^{a} (100)	8.77 ± 0.88^{b} (91)	9.67 ± 1.36^{a} (100)	6.89 ± 1.26^{b} (71)	9.04 ± 1.40^{a} (100)	7.30 ± 1.50^{b} (81)
Insulin (*pmol/L*)	354.09 ± 73.83^{a} (100)	356.67 ± 72.32^{a} (101)	364.13 ± 78.21^{a} (100)	379.27 ± 78.28^{a} (104)	373.32 ± 82.30^{a} (100)	373.03 ± 85.31^{a} (100)
Glucometer data (blood)						
Fasting glucose (*mmol/L*)	8.85 ± 2.15^{a} (100)	8.60 ± 1.88^{a} (97)	9.02 ± 2.86^{a} (100)	7.62 ± 2.35^{a} (84)	9.16 ± 3.27^{a} (100)	8.21 ± 2.94^{a} (90)
Glucose 1/2 hr. before dinner (*mmol/L*)	9.71 ± 3.67^{a} (100)	9.21 ± 2.29^{a} (95)	9.33 ± 3.05^{a} (100)	8.07 ± 2.72^{a} (86)	9.73 ± 3.46^{a} (100)	8.05 ± 3.01^{a} (83)

[1] Feeding period was 60 days for each rice bran fraction and 30 days for washed out (cellulose).
[2] Time of drawing blood was 0800. The subjects were fasted for 12 hr before samples were taken.
* X ± SD (mean ± standard deviation).
[3] Percentage with respect to baseline values are in parentheses.
[a,b] Values in a row not sharing a common superscript letter are significantly different, $P < 0.05$.

(# 356-A, 340 nm), apolipoprotein B (# 357, 340 nm) and obtained from Sigma Chemical Co. St. Louis, MO. Platelet Factor 4 (PF4) was determined by using Elisa Kit for PF4 (# [601], American Bioproduct, North Jefferson Road, Parsippany, New Jersey) and a radioimmunoassay kits were used to determine thromboxane B_2 (# TxB_2, Chemicon International, El Segundo, CA, USA), and Lp(a) levels (Terumo Medical Corporation, Diagnostic Division (Elkton, MD USA). The levels of serum insulin were estimated by using a Double Antibody RIA Kit (Ventrex Laboratories, Inc. 217 Read Street, P. O. Box. 9731, Portland, USA), and glucagon, by using RSL Glucagon Kit (ICN Biomedicals, Inc., Diagnostic Division, Costa Mesa, CA).

2.2. Preparation of sachets of stabilized rice bran, rice bran water solubles, and rice bran fiber concentrates

The sachets (10 gm of each) of stabilized rise bran (A), or rice bran water solubles (B) or rice bran fiber concentrates (C) were supplied by "The RiceX Company," 1241 Hawk's Flight Court, El Dorado Hills, CA. The rice bran water solubles and rice bran fiber concentrates were prepared by making a slurry of stabilized rice bran (20% to 25%) in water, followed by treatments with carbohydrate-cleaving enzymes (dextranase, or α-amylase or maltase) to convert starch to dextrins and heating in a steam injection cooker at 70–90°C. The enzyme and heated slurry was then pumped to a horizontal centrifuge. Following centrifugation, the lower liquid portion was pumped into a drum dryer, dried, and ground to a fine powder yielding the rice bran water solubles (B). The upper portion was dried on a belt dryer and ground to a fine powder yielding rice bran fiber concentrates (C) [18; US Patent 6,126,943 (Oct. 3, 2000); US Patent 6,303,585 B1 (Oct. 16, 2001)].

2.3. Study population

Subjects with clinically established Type I or Type II diabetes were recruited from Armed Forces military and

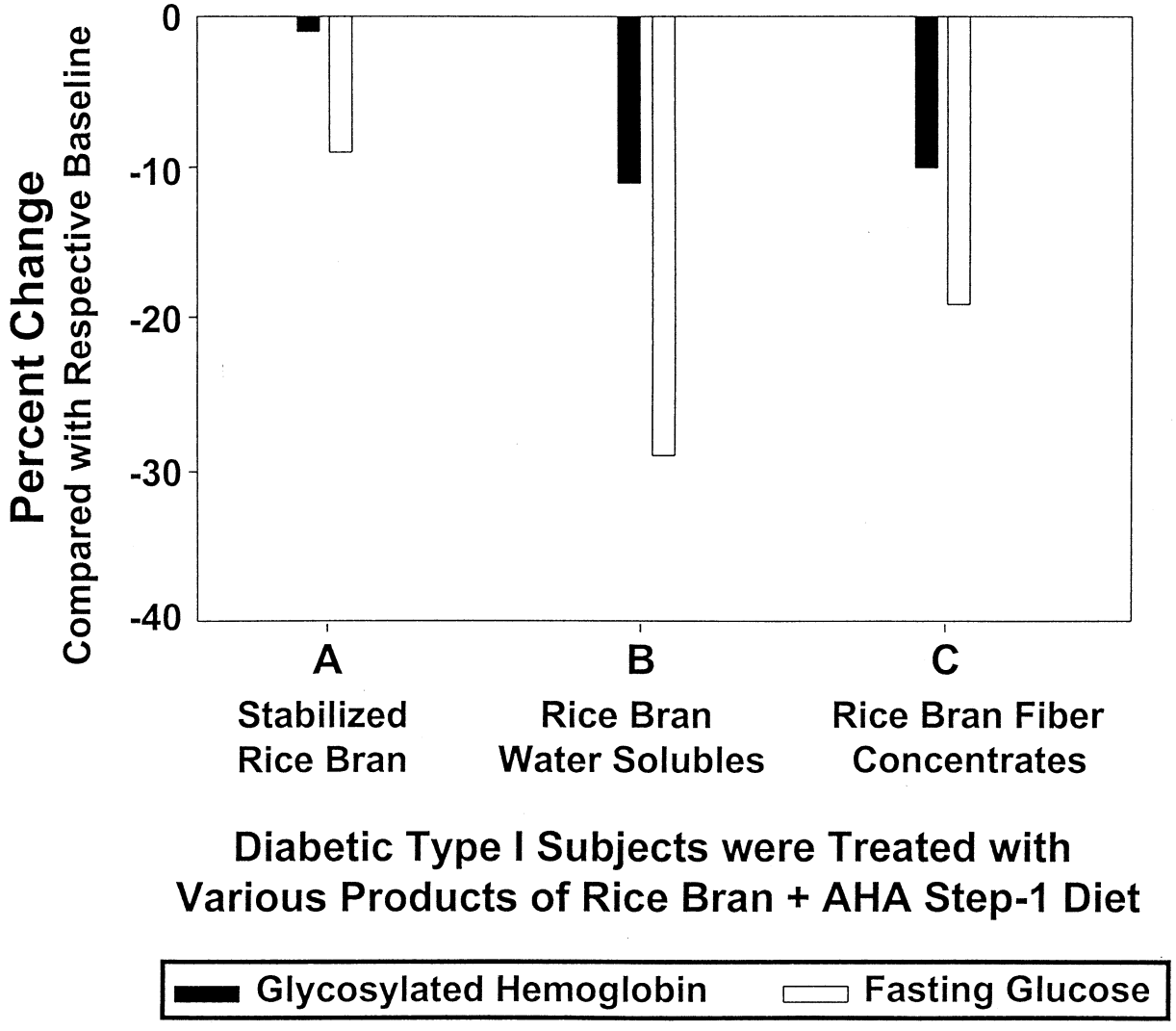

Fig. 2. The main highlights of comparative effects of various stabilized rice bran (A), its subfractions-rice bran water solubles (B), and rice bran fiber concentrates (C) on serum glycosylated hemoglobin and fasting glucose levels in Type I (insulin-dependent diabetes mellitus, IDDM) human subjects.

Table 5
Effects of stabilized rice bran and its subfractions on lipid parameters in Type I diabetes (Insulin-Dependent Diabetes Mellitus, IDDM) human subjects[1]

Lipid parameters[2] (serum)	Stabilized rice bran (A; $n = 22$)		Rice bran water solubles (B; $n = 26$)		Rice bran fiber concentrates (C; $n = 20$)	
	Before baseline	After (A)	Before cellulose + baseline	After (B)	Before cellulose + baseline	After (C)
Total cholesterol (*mmol/L*)	4.70 ± 0.68[a,*] (100)	4.66 ± 0.75[a] (99)	4.51 ± 0.57[a] (100)	4.30 ± 0.54[a] (95)	4.80 ± 0.81[a] (100)	4.34 ± 0.82[b] (90)
LDL-cholesterol (*mmol/L*)	3.56 ± 0.65[a] (100)	3.47 ± 0.68[a] (97)	3.38 ± 0.55[a] (100)	3.16 ± 0.52[b] (93)	3.48 ± 0.65[a] (100)	2.94 ± 0.56[b] (84)
Apolipoprotein B (*g/L*)	0.88 ± 0.07[a] (100)	0.86 ± 0.06[a] (98)	0.86 ± 0.05[a] (100)	0.82 ± 0.06[b] (93)	0.84 ± 0.05[a] (100)	0.76 ± 0.06[b] (90)
Triglycerides (*mmol/L*)	1.53 ± 0.12[a] (100)	1.52 ± 0.13[a] (100)	1.51 ± 0.16[a] (100)	1.47 ± 0.14[a] (97)	1.47 ± 0.15[a] (100)	1.36 ± 0.10[b] (93)
HDL-cholesterol (*mmol/L*)	0.97 ± 0.19[a] (100)	0.97 ± 0.97[a] (100)	1.00 ± 0.16[a] (100)	0.98 ± 0.16[a] (98)	1.02 ± 0.16[a] (100)	1.02 ± 0.16[a] (100)
Apolipoprotein A-I (*g/L*)	1.16 ± 0.03[a] (100)	1.17 ± 0.03[a] (100)	1.15 ± 0.04[a] (100)	1.15 ± 0.04[a] (100)	1.16 ± 0.03[a] (100)	1.16 ± 0.03[a] (100)

[1] Feeding period was 60 days for each rice bran fraction and 30 days for washed out (cellulose).
[2] Time of drawing blood was 0800. The subjects were fasted for 12 hr before samples were taken.
* X ± SD (mean ± standard deviation).
[3] Percentage with respect to baseline values are in parentheses.
[a,b] Values in a row not sharing a common superscript letter are significantly different, $P < 0.05$.

civilian personel headquartered in Rawalpindi, Pakistan. Eligible subjects include male and non-pregnant female patients, between the ages of 20–65 years within ±20% ideal body weight. Excluded were patients with impaired kidney function (creatinine clearance <40 ml/min), impaired liver function (an elevated serum glutamate pyruvate or glutamate oxaloacetate transaminase activity or an elevated blood urea nitrogen), cardiac problems, uncontrolled hypertension (systolic blood pressure >200 mm Hg, and diastolic blood preessure >110 mm Hg), with elevated fasting serum triglycerides (<1.75 *mmol/L*) and total cholesterol levels (<4.95 *mmol/L*). The physical profile of the 45 subjects recruited for the Type I group, age 35 ± 11 years, height 163 ± 10 cm), weight (70 ± 10 Kg), and body mass index (26 ± 4) differed as anticipated in some respects (age, weight) from that of the 41 subjects recruited for the Type II group (age 48 ± 12 years), height (162 ± 8 cm), weight (68 ± 10 Kg), and body mass index (26 ± 4). Type I subjects were continued on prescribed medication (insulin: 0.1–0.6 ml/day of 100 IU/ml). All subjects continued throughout the study on physician-recommended diets similar to American Heart Association (AHA) Step-1 diet (restricted intake of saturated fat <10%, total fat <30% and cholesterol <7.76 *mmol/L*). All subjects were instructed to avoid alcoholic beverages and to maintain a regular routine during the course of study. Subjects met regularly in small groups for dietary counseling, discussions of the relationship between diet and risk for diabetes and cardiovascular disease. Each subject was given the telephone number of a staff contact person. All subjects signed an informed-consent form approved by the Institutional Review Board of the Armed Forces Institute of Pathology, Rawalpindi, Pakistan. This study was carried out under a FDA approved IND number 30906.

2.4. Experimental design

The study was designed as a double blind, cross-over clinical trial of the three rice bran products, stabilized rice bran (A), rice bran water solubles (B), and rice bran bran fiber concentrates (C), each administered as two 10-g sachets/day for an 8-week period with a 4-week washout period between exposure to each product. For 4-weeks preceding the start of the study and during the washout period, the subjects were given 2 sachets/day of cellulose (10 g each). Subjects were randomly assigned to one of 6 potential product sequences (A, B, C; A, C, B; B, C, A; B, A, C; C, A, B; C, B, A) with a washout between each product phase as outlined in Figure 1. The sachets were self-administered with a glass of water or juice, 1 sachet before breakfast and 1 before dinner. The subjects were instructed in the use of a Glucometer to measure (by poking the finger) their blood glucose values prior to taking the sachets; subjects kept records of their blood glucose values. Hypoglycemic episodes were managed by reducing medications and/or dietary management as recommended by the study physician. Fasting (12-hr) blood (capillary) samples were drawn from each subject after each 4-week cellulose regimen and after each 8-week product regimen. Blood samples were analyzed on site (Armed Forces Institute of Pathology, Rawalpindi, Pakistan) for hemoglobin, glycosylated hemoglobin and glucose levels. Serum samples were held at –70°C; coded samples were shipped to Advanced Medical Research, Madison, WI, where analyses for individual parameters, e.g. insulin, glucagon, total cholesterol, LDL-cholesterol, apolipoprotein B, Lp(a), HDL-cholesterol, apolipoprotein A-I, triglycerides, thromboxane B2 and PF4 levels were performed in single runs, as described in detail recently [24–26], in an effort to reduce analytical variation.

Table 6
Effects of stabilized rice bran and its subfractions on glycemic parameters in Type II diabetes (Noninsulin-Dependent Diabetes Mellitus, NIDDM) human subjects[1]

Glycemic parameters[2]	Stabilized rice bran (A; $n = 23$)		Rice bran water solubles (B; $n = 31$)		Rice bran fiber concentrates (C; $n = 26$)	
	Before baseline	After (A)	Before cellulose + baseline	After (B)	Before cellulose + baseline	After (C)
Phlebotomy data (serum)						
Glycosylated hemoglobin (%)	$10.22 \pm 1.84^{a,*}$ (100)[3]	10.03 ± 2.16^{a} (98)	10.69 ± 1.97^{a} (100)	9.05 ± 1.68^{a} (85)	10.70 ± 2.00^{a} (100)	9.51 ± 2.06^{a} (89)
Fasting glucose (*mmol/L*)	8.78 ± 1.41^{a} (100)	7.90 ± 1.11^{b} (90)	8.79 ± 0.97^{a} (100)	5.91 ± 1.37^{b} (67)	8.07 ± 0.97^{a} (100)	6.31 ± 1.45^{b} (78)
Insulin (*pmol/L*)	354.59 ± 62.06^{a} (100)	358.61 ± 63.21^{a} (101)	347.84 ± 68.81^{a} (100)	360.97 ± 68.81^{a} (104)	354.80 ± 60.70^{a} (100)	358.32 ± 61.35^{a} (101)
Glucometer data (blood)						
Fasting glucose (*mmol/L*)	6.67 ± 1.64^{a} (100)	6.76 ± 1.61^{a} (101)	7.13 ± 1.77^{a} (100)	6.56 ± 1.50^{a} (90)	7.17 ± 2.10^{a} (100)	6.42 ± 1.22^{a} (90)
Glucose 1/2 hr. before dinner (*mmol/L*)	6.67 ± 2.02^{a} (100)	7.21 ± 1.54^{a} (108)	7.20 ± 1.89^{a} (100)	6.86 ± 1.59^{a} (95)	7.47 ± 2.80^{a} (100)	6.71 ± 1.62^{a} (90)

[1] Feeding period was 60 days for each rice bran fraction and 30 days for washed out (cellulose).
[2] Time of drawing blood was 0800. The subjects were fasted for 12 hr before samples were taken.
* X ± SD (mean ± standard deviation).
[3] Percentage with respect to baseline values are in parentheses.
[a,b] Values in a row not sharing a common superscript letter are significantly different, $P < 0.05$.

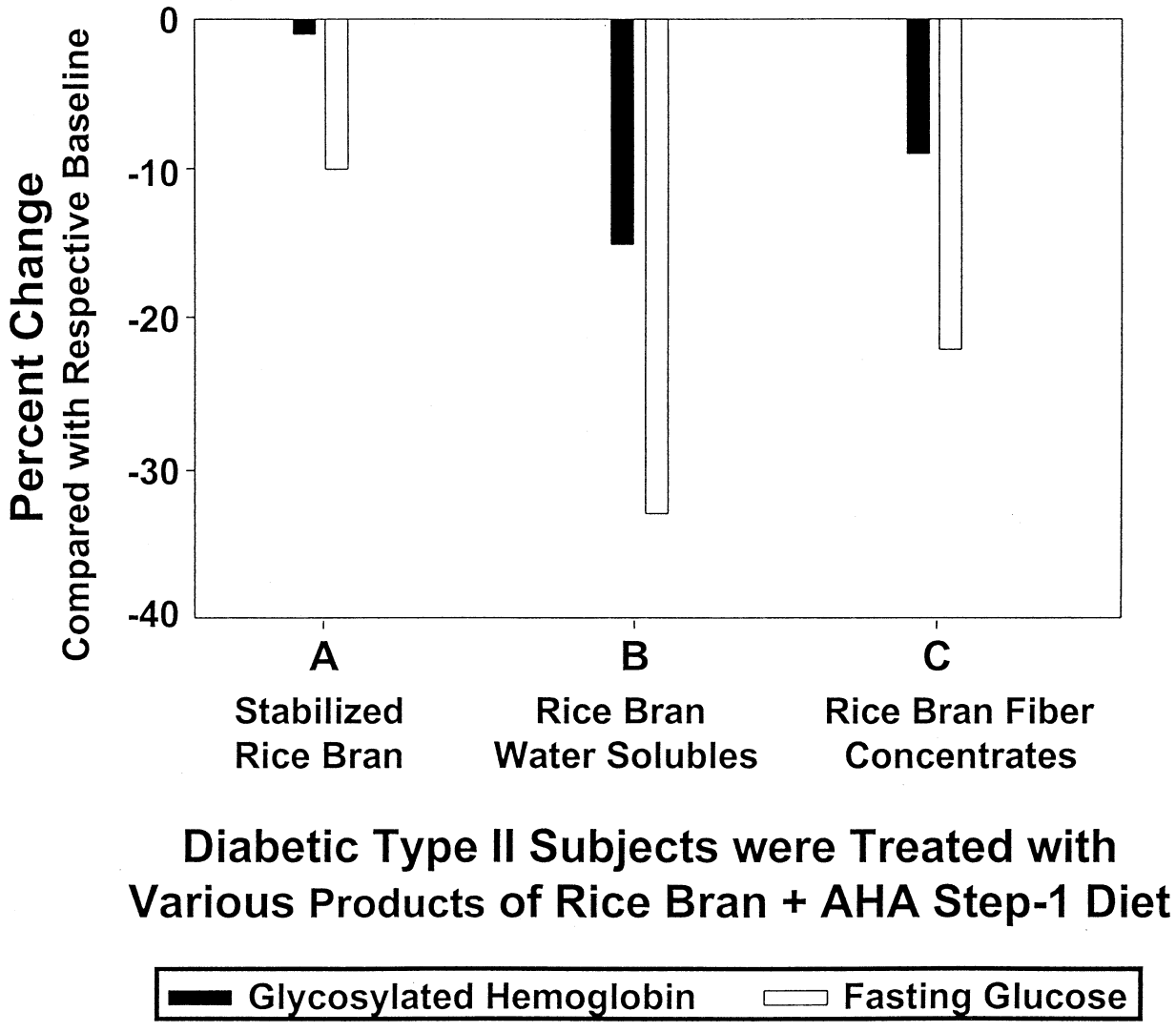

Fig. 3. The main highlights of comparative effects of various stabilized rice bran (A), its subfractions-rice bran water solubles (B), and rice bran fiber concentrates (C) on serum glycosylated hemoglobin, and fasting glucose levels in Type II (noninsulin-dependent diabetes mellitus, NIDDM) human subjects.

Subject weights and adherence to medications were monitored. Diet records and 24-hr recalls were analyzed (Nutrition Co-ordinating Center, University of Minnesota, Minneapolis, MN); if required, subjects were individually counseled to modify food intake to meet the goals of the AHA Step-1 diet or to maintain weight.

2.5. Analyses of serum cholesterol values and different lipid parameters

The analyses of the coded samples were performed as described in detail recently at Advanced Medical Research, Madison, WI, [24–26]. The serum total cholesterol, HDL-cholesterol, and triglycerides concentrations were estimated with reagent kits from Sigma Chemical Co. (St. Louis, MO).

Serum LDL-cholesterol was precipitated from 200 μl of serum with 25 μl of a mixture of 9.7 mM phosphotungstic acid and 0.4 M $MgCl_2$. The preparation was mixed for 10 min at room temperature and then centrifuged at 12,000 x g for 19 min. The supernatant was decanted and analyzed for HDL-cholesterol. The precipitate was dissolved in 200 μl of 0.1 M sodium citrate and the concentration of LDL-cholesterol was estimated as described for total cholesterol [27].

Serum apolipoprotein A-1 (apo A-1), and apolipoprotein B (apo B) concentrations were determined by radioimmunoassay using kits from Sigma Chemical Co. (St. Louis, MO) in the placebo and treatment groups. Similarly, plasma/serum LP(a), platelet factor 4, thromboxane B_2 (TxB_2), insulin, and glucagon concentrations were determined by various radioimmunoassay using diagnostic kits for these parameters [24–26]. All assays for each subject were carried out at the same time under similar conditions to minimize standard deviation. To determine if subjects have

complied with the request to fast prior to blood drawing, aliquots were used at the site to detect the presence of chylomicrons.

2.6. Statistical analysis

The data were analyzed by using the GLM procedure of SAS (Statistical Analysis System) for personal computers to test the study hypothesis. Treatment-mediated differences for serum glycemic and lipid parameters were identified with ANOVA, using changes from baseline values (0-time) to the end of each treatment. When the F test indicated a significant effect, the differences between the means were analyzed by a Fisher's Protected Least Significance Difference (LSD) test (Abacus Concepts 1992) [28]. Data are reported as mean ± SD in the text. The statistical significance level was set at $P < 0.05$.

3. Results

The stabilized rice bran (A) contains a mixture of complex carbohydrates (51.0%), protein (14.5%) and microcomponents (<1.1%; vitamin E, γ-oryzanols, terpenes and polyphenols The rice bran water solubles (B) consists of simple carbohydrates as dextrins (57.5%), protein (7.5%), and rice bran fiber concentrates (C) consists of carbohydrates (52.5%), protein (20.5%) and fibers (42%). Both of these subfractions of stabilized rice bran also contain the same microcomponents as in stabilized rice bran (Table 1) [18]. The number of subjects completed the study of each rice bran product (A, B, C) of Type I and Type II are reported in Table 2. A total of 11 Type I and 18 Type II subjects completed all phases of the study. A total of 45 subjects, 22 Type I and 23 Type II received stabilized rice bran (A). The rice bran water solubles (B) was administered to 57 subjects, 26 Type I and 31 Type II, and subjects, 20 Type I and 26 Type II, received the rice bran fiber concentrates (C) (Table 2). The effects of rice bran products (A, B, C) on the consumption of protein, fat, and carbohydrates, as well as other physical characteristics of both type of diabetic subjects are reported in Table 3. Subjects in each group of various rice products (A, B, C) treatments showed no significant change in any parameters or gain in body weight throughout the study (Table 3).

3.1. Effects of stabilized rice bran and its subfractions on glycemic parameters in Type I diabetes human subjects

Rice bran water solubles (B) and rice bran fiber concentrates (C) fed to diabetic Type I subjects for 60 days caused a reduction of 11% and 10% in serum glycosylated hemoglobin compared to baseline values (Table 4). The stabilized rice bran (A) did not affect serum glycosylated hemoglobin in Type I subjects (Table 4). The fasting serum glucose levels lowered significantly with products (B), 29% ($P < 0.01$), and (C), 19% ($P < 0.03$) at the end of the feeding period (Table 4). The product (A) showed a decrease of 9%. The Glucometer estimations (by each subject) of serum fasting glucose and ½ hr before dinner glucose levels with products (B) and (C) showed a reduction of 16% , 14% and 11%, 17%, respectively compared to their baseline values (Table 4). Some of these impressive decreases are not statistically significant due to large standard deviation (>20%). The main highlights of the comparative effects of various stabilized rice bran, and its subfractions on glycosylated hemoglobin and fasting glucose levels are summarized in Figure 2. The serum insulin level showed a 4% increase with rice bran water solubles (Table 4). There was no effect on the levels of glucagon with any rice bran fractions (data not shown).

3.2. Effects of stabilized rice bran and its subfractions on lipid parameters in Type I diabetes human subjects

Serum total cholesterol, LDL-cholesterol, apolipoprotein B, and triglycerides levels were decreased with rice bran water solubles (B), 5% 7%, 7% ($P < 0.004$), and 3% (not significant) and with rice bran fiber concentrates (C), 10%, 16%, 10%, and 7% ($P < 0.001$), respectively, compared to their baseline values in Type I human subjects (Table 5). The stabilized rice bran (A) showed not significant decreases in these parameters compared to their baseline values (Table 5). The serum HDL-cholesterol and apolipoprotein A-I levels (Table 5) were not affected, whereas, Lp(a), thromboxane B_2 and platelet factor 4 showed not significant decreases with all the rice bran products (A, B, C, Data not shown).

3.3. Effects of stabilized rice bran and its subfractions on glycemic parameters in Type II diabetes human subjects

The stabilized rice bran (A), rice bran water solubles (B), and rice bran fiber concentrates (C) fed to diabetic type II human subjects for 60 days showed similar results as observed in Type I subjects. The glycosylated hemoglobin decreased 15% and 11%, respectively, with rice bran products B and C compared to their respective baseline values (Table 6). However, fasting glucose levels decreased significantly 10%, 33% and 22%, respectively ($P < 0.05$) with all three rice bran products (A, B, C) in Type II human subjects compared to their baseline values (Table 6). The main highlights of the comparative effects of various stabilized rice bran, and its subfractions on glycosylated hemoglobin and fasting glucose levels are summarized in Figure 3. The serum insulin level was increased 4% compared to baseline value with rice bran water solubles (B) only. Similarly, the serum glucose levels of fasting and ½ hr. before dinner monitored by Glucometers by Type II human subjects when fed rice products A, B, C for 60 days showed statistically not significant decreases in these parameters

Table 7
Effects of stabilized rice bran and its subfractions on lipid parameters in Type II diabetes (Noninsulin-Dependent Diabetes Mellitus, NIDDM) human subjects[1]

Lipid parameters[2] (serum)	Stabilized rice bran (A; $n = 23$)		Rice bran water solubles (B; $n = 31$)		Rice bran fiber concentrates (C; $n = 26$)	
	Before baseline	After (A)	Before baseline + cellulose	After (B)	Before baseline + cellulose	After (C)
Total cholesterol (*mmol/L*)	$4.73 \pm 0.43^{a,*}$ (100)[3]	4.47 ± 0.44^{b} (95)	4.68 ± 0.50^{a} (100)	4.42 ± 0.24^{b} (94)	4.810 ± 0.59^{a} (100)	4.26 ± 0.51^{b} (88)
LDL-cholesterol (*mmol/L*)	3.78 ± 0.56^{a} (100)	3.49 ± 0.31^{b} (92)	3.70 ± 0.55^{a} (100)	3.40 ± 0.35^{b} (92)	3.79 ± 0.47^{a} (100)	3.23 ± 0.37^{b} (85)
Apolipoprotein B (*g/L*)	0.96 ± 0.17^{a} (100)	0.94 ± 0.16^{a} (98)	0.95 ± 0.17^{a} (100)	0.92 ± 0.12^{a} (97)	0.95 ± 0.17^{a} (100)	0.86 ± 0.14^{b} (91)
Triglycerides (*mmol/L*)	1.62 ± 0.13^{a} (100)	1.57 ± 0.14^{a} (97)	1.57 ± 0.14^{a} (100)	1.53 ± 0.12^{a} (98)	1.61 ± 0.14^{a} (100)	1.48 ± 0.10^{b} (92)
HDL-cholesterol (*mmol/L*)	0.94 ± 0.21^{a} (100)	0.94 ± 0.21^{a} (100)	0.89 ± 0.15^{a} (100)	0.89 ± 0.15^{a} (100)	0.87 ± 0.14^{a} (100)	0.87 ± 0.15^{a} (100)
Apolipoprotein A-I (*g/L*)	1.16 ± 0.04^{a} (100)	1.16 ± 0.04^{a} (100)	1.18 ± 0.05^{a} (100)	1.18 ± 0.04^{a} (100)	1.17 ± 0.03^{a} (100)	1.18 ± 0.03^{a} (100)

[1] Feeding period was 60 days for each rice bran fraction and 30 days for washed out (cellulose).
[2] Time of drawing blood was 0800. The subjects were fasted for 12 hr before samples were taken.
* X ± SD (mean ± standard deviation).
[3] Percentage with respect to baseline (before) values are in parentheses.
[a,b] Values in a row not sharing a common superscript letter are significantly different, $P < 0.05$.

compared to their respective baseline values due to large standard deviation (Table 6).

3.4. Effects of stabilized rice bran and its subfractions on lipid parameters in Type II diabetes human subjects

Serum total cholesterol and LDL-cholesterol in Type II human subjects fed rice bran products show significant ($P < 0.05 \sim 0.001$) decreases with A (5%, 8%), B (6% 8%), and C (12% 15%) compared to their beaseline values (Table 7). The apo B and triglycerides levels decrease (9% and 8%) with product C only. The plasma/serum HDL-cholesterol, apolipoprotein A-I (Table 7), Lp(a), platelet factors 4, and thromboxane B_2 (data not shown) were not affected after feeding rice bran products (A, B, or C) to Type II human subjects.

Six Type I subjects out of 26 subjects after taking rice bran water solubles (B) for 60 days reduced their doses of insulin at least 50% and five subjects out of 20 subjects on rice bran fiber concentrates (C) reduced 30% to 40%. In the case of Type II subjects, 4 out of 31 subjects after taking rice bran water solubles (B) for 60 days reduced their intake of hypoglycemic drugs more than 60% and four subjects out of 26 subjects on rice bran fiber concentrates (C) also reduced more than 60% of taking the hypoglycemic drugs.

4. Discussion

The results of the present study clearly demonstrate that rice bran water solubles (B) and rice bran fiber concentrates (C) are more effective in reducing the serum glucose levels in both types (I and II) of diabetes in humans as compared to stabilized rice bran (A). The reduction of glycosylated hemoglobin by these subfractions is very useful for the control of blood glucose levels in these subjects. A number of investigators have reported that these biological effects might be due to the synergistic effects of the multiple bioactive microcomponents present in these subfractions (B and C) of stabilized rice bran (Table 8) [18–23,29]. All these microcomponents, including antioxidants such as tocopherols (vitamin E), tocotrienols, γ-oryzanols, polyphenols (ferulic acid, α-lipoic acid, *p*-sinapic acid) are present in different concentrations in all three rice bran fractions and might be able to maintain glucose levels by exerting their effects on glucose absorption, utilization, and excretion [18–23,29]. These compounds are free radical scavengers and can improve the complications of diabetes such as glycation, glycoxidation, atherosclerosis and hyperlipidemia [18]. Moreover, the positive effects of vitamin E to correct the complications of diabetes due to its antioxidant property have been reported [30–33]. Among polyphenols, α-lipoic acid acting as lipoate has gained attention as a potential therapeutic agent for diabetes-induced complications. Non-enzymatic glycation of proteins has been found to increase in a variety of proteins in diabetic patients. Lipoate prevents glycation and subsequent glucose-induced structural modification of various proteins [34,35].

As mentioned above, the microcomponents of rice bran products B and C might be responsible for lowering and sustaining the glucose levels in both types of diabetes, but it is difficult to attribute the effectiveness of rice bran water solubles (B) and fiber concentrates (C) solely to their microcomponents. Most of these microcomponents are present in much higher concentrations in stabilized rice bran (A), which contains 23% to 16% more of these microcomponents than rice bran water solubles and rice bran fiber

Table 8
Composition of microcomponents including antioxidants of stabilized rice bran, rice bran water solubles, and rice bran fiber concentrates

Carotenoids 0.9–1.6 ppm	**Other antioxidants (ppm)**	**γ-Oryzanols 2200–3000 ppm**
α-Carotene	Inositol (1100–1400)	Cycloatenyl ferulate
β-Carotene	Myoinositol (1000–1200)	Campesteryl ferulate
Lycopene	Choline (930–1150)	Stigmasteryl ferulate
Lutein	Phytates (1500–1710)	β-Sitosteryl ferulate
Zeaxanthine	Biotin (0.1–0.22)	24-Methylene cyclartanyl ferulate
Tocopherols/Tocotrienols (Vitamin E) 210–440 ppm	**Vitamin-B Complex (ppm)**	**Phospholipids**
α-Tocopherol	Niacin (370–660)	Phosphatidylcholine
β-Tocopherol	Pantothenic acid (36–50)	Phosphatidylethanolamine
γ-Tocopherol	Pyridoxin (29–42)	Lysophosphatidylcholine
δ-Tocopherol	Thiamin (22–31)	Lysophosphatidylethanolamine
α-Tocotrienol	Riboflavin (2.2–3.5)	
β-Tocotrienol		**Polysaccharides**
γ-Tocotrienol	**Phytosterols**	
δ-Tocotrienol	**2230–4400 ppm**	Cycloartenol ferulic acid glycoside
Desmethyl Tocotrienol		Diferulic acid complex
Didesmethyl Tocotrienol	β-Sitosterol	Diferulic acid + 3 Glucose + 2-Calcium complex
	Campesterol	
Polyphenols 305–390 ppm	Stigmasterol	**Amino Acids**
	Δ5 Avinsterol	
Ferulic acid	Δ7 Stigmasterol	Arginine (10800 ppm)
α-Lipoic acid		Histidine (3800 ppm)
Methyl ferulate	Gramisterol	Methionine (2500 ppm)
p-Coumaric acid	Citrostdienol	Tryptophan (2100 ppm)
p-Sinapic acid	Obtusifoliol	Cystein (336–448 ppm)
Quercetine	Branosterol	Cystine (336–448)
Isovitexin	28-Homotyphasterol	
Proanthocyanidins	28-Homosteasteronic acid	
Caffiec acid	6-Deoxycastasterone	**Minerals**
cinnamic acid	β-Amyrin	
	Enzymes	Magnesium (6250–8440 ppm)
		Calcium (303–500 ppm)
	Glutathione peroxides	Phosphorous (14700–17000 ppm)
	Methionine reductase	
	Superoxidase dismutase	
	Polyphenol oxidase	
	Coenzyme Q10	

concentrates (Table 1). Stabilized rice bran (A) did not lower significantly serum glucose levels in these diabetic human subjects. Therefore, other factor(s) in rice bran water solubles (B) and rice bran fiber concentrates (C) must be responsible for the reductions in glucose levels in both types (I and II) of diabetes subjects.

One of the two possible factors responsible for the glucose reduction in diabetes might be the "protein" of the enzyme-treated, and heated products (B and C) of the stabilized rice bran. Although the percentage of protein products B (7.5%) and C (20.5%) varies from the stabilized rice bran (14.5%), the configuration of proteins of these subfractions might be changed stereospecifically due to enzyme-, and heat-treatment of stabilized rice bran (Table 1). Recently, Vlassara *et al.* [36,37] has reported that a number of proteins undergo a series of nonenzymatic reactions with glucose over time to form advanced glycosylation end products (AGEs), which are implicated in the multiorgan complications of diabetes through macrophages [37]. Macrophages have a receptor that recognizes the AGE moiety and mediates the uptake and degradation of AGE protein. This glucose-modified protein (AGEs) signals macrophages to secrete tumor necrosis factor (TNF), interleukin-1 (IL-1), and other cytokines, which in turn influence the degradation and proliferation of tissue components [38]. An imbalance of this system in certain states, such as in diabetes where accelerated AGE formation occurs as a result of high glucose concentration, causes an increase accumulation of AGE, which may explain in part the excessive proliferative response characteristic of several diabetic tissues [36–39]. The protein of rice bran water solubles and rice bran fiber concentrates might inhibit the formation of AGE products.

The second possible factor for this reduction of glucose in diabetes by products B and C of stabilized rice bran could

be due to small peptide(s). The enzyme-, and heat- (>70°C) treatments of stabilized rice bran resulted not only the conversion of complex carbohydrates (starch) into simple-dextrins but also caused degradation of protein into smaller molecular weight peptides stereospecifically. The important roles played by peptide(s) in a number of different biological functions has been reported [40–42]. The most important amino acid of the protein/peptides, which plays an important role in diabetes, is L-arginine, which is found in highest levels in the protein/peptides of these subfractions (Table 8) [42,43]. A 37-amino acid peptide has been isolated from the islet amyloid of patients with Type II patient [41]. The small molecular weight peptides of these two fractions may possibly play an important role in the control of blood glucose levels in both types of diabetic human subjects. We are presently isolating these small molecular weight peptides from rice bran water solubles by extracting with a mixture of acetonitrile and water (30 : 70) or acetone, and purifying by SDS-PAGE. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) will be used to establish the structures of these peptides, and amino acid sequence of the peptide/peptides will be determined.

The effect of rice bran water solubles and fiber concentrates on lowering serum total cholesterol, LDL-cholesterol, triglycerides and apolipoprotein B levels was not as significant as observed with glucose levels in these diabetic human subjects. This poor response of products B and C might be due to the fact that all of the subjects were not hypercholesterolemic. The rice bran fiber concentrates reduced the lipid parameters better than rice bran water solubles due to its high fiber contents (>40%). The bile acids bind with fiber, thereby lowering serum total cholesterol and triglycerides levels, thus helping in the cholesterol metabolism.

This is the first report that demonstrates that rice bran water solubles is more effective in lowering serum glucose levels, and rice bran fiber concentrates is more effective in lowering serum total cholesterol, LDL-cholesterol and apolipoprotein B levels in both types of (I and II) diabetes human subjects. The reduction of glycosylated hemoglobin, blood glucose (as shown in Figures 2, and 3) and a slight increase in insulin levels can be controlled human diabetes by consuming rice bran water solubles. Another important finding of the present study is that approximately 25% of the subjects in both diabetes group decreased their daily injection of insulin and intake of hypoglycemic drugs (30% –60%) after the consumption of rice bran products B and C for 60 days. Thus these natural products may be used as nutritional supplements for the control of diabetes mellitus Types I and II in human subjects.

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Abbreviations

AHA Step-1 diet	American Heart Association Step-1 diet
Apo A-1	apolipoprotein A-1
Apo B	apolipoprotein B
HDL-cholesterol	high density lipoprotein cholesterol
LDL-cholesterol	low density lipoprotein cholesterol
PF 4	platelet factor 4
RBFC	rice bran fiber concentrates (product C)
RBWS	rice bran water solubles (product B)
SRB	stabilized rice bran (product A)
Type I	Insulin-Dependent-Diabetes Mellitus (IDDM)
Type II	NonInsulin-Dependent Mellitus (NIDDM)
TxB_2	Thromboxane B_2

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