EFFECT OF RICE BRAN AND SUNFLOWER OIL BLEND: ON BLOOD CHOLESTEROL AND BLOOD TRIGLYCERIDES IN HUMAN SUBJECTS WITH HYPERLIPIDEMIA AND NORMOLIPIDEMIA

Prof. Adrian Kennedy

Prof. N Naidu

Dr. Shyamala Sesikiran

Ms. Nidhi Lakra

LIFESTYLE UNIT, APOLLO HEALTH STREET LIMITED

1st Floor Academic Block, Apollo Hospitals Campus, Jubilee Hills, Hyderabad -33

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Abbreviations Used

ADP = Adenosine Di-phosphate

CAD = Coronary Artery Disease

ECG = Electro Cardiogram

GNO = Groundnut Oil

GTT = Glucose Tolerance Test

HDL = High Density Lipoprotein

LDL = Low Density Lipoprotein

MUFA = Mono unsaturated Fatty acids

NIN = National Institute of Nutrition

PHS = Preliminary Health Scan.

PUFA = Polyunsaturated Fatty acids

RBO = Rice-bran Oil

SFA = Saturated Fatty acids

SFO = Sunflower Oil

SE = Standard Error

TC = Total Cholesterol

Tgl = Triglycerides

VLDL = Very Low density Lipoprotein

New Blend = 80:20 blend of Ricebran and Sunflower oils with minimum 5000 ppm Oryzanol

Effect of Rice Bran and Sunflower Oil Blend:

On Blood Cholesterol & Blood Triglycerides,

In Human Subjects with Hyperlipidemia & Normolipidemia.

Prof. Adrian Kennedy, (PhD) USA. Lifestyle Unit, Apollo Hospitals, Hyderabad.

Prof. N. Naidu (Retd.) Statistician National Institute of Nutrition, Hyderabad,

Dr. Shyamala Sesikeran, M.D. Blood Bank, Apollo Hospitals, Hyderabad, and

Ms. Nidhi Lakra Msc. (Food & Nutrition) Apollo Hospitals, Hyderabad.

Abstract

The hypolipidemic action of dietary Rice bran + Sunflower oil blend in 80:20 ratio with minimum 5000 ppm of Oryzanol and a defined fatty acid profile (henceforth referred to as new blend), was investigated in human subjects with hyperlipidemia. Thirty-two subjects with either high serum cholesterol or high serum triglycerides were divided into two groups of sixteen each for this 60-day trial. During the first 30 days the first group of sixteen consumed their regular oil (sunflower/groundnut), while the second group of sixteen were given the blend for daily use. During the next 30 days the first group of sixteen were switched to the blend, whereas the second group of sixteen were reverted back to their regular oils. Blood samples were drawn from all subjects on day 0, 15, 30, 45 and 60. There was a statistically significant reduction (at 95% confidence level) in serum Total cholesterol, Low Density Lipoprotein cholesterol, Very Low Density Lipoprotein cholesterol & Serum Triglyceride levels in the subjects after using the blend, and no significant change in High Density Lipoprotein cholesterol, making the blend, an appropriate oil for consumption in individuals with Hyperlipidemic Metabolism.

Another group of 16 subjects with normal total cholesterol (180 – 200 mg/dl) or total triglycerides (<150 mg/dl) also consumed the new blend for first 30 days and then switched to their regular oils (either sunflower or groundnut) for next 30 days. This normolipidemic group also showed statistically significant reduction in total cholesterol, Very Low Density Lipoprotein Cholesterol and total triglycerides after using blend oil for 30 days, making the blend, an excellent oil for consumption in order to reduce risk of Hyperlipidimia in Normolipidemic persons.

Objective

Besides dietary intake of oils, several lifestyle related factors including inappropriate diet, sedentary life, lack of exercise, obesity, stress, alcohol, smoking & family predisposition are known to affect blood cholesterol & blood triglycerides in humans. The objective of this study was to isolate the effect of rice bran and sunflower oil blend (in 80:20 ratio with 5000 ppm of Oryzanol), on human Hyperlipidemic and Normolipidemic subjects after ensuring that all other known predisposing lifestyle factors as mentioned above remains unchanged in all subjects during the period of trial, such that the only factor that influenced blood cholesterol & blood triglycerides in subjects was the oil administered during the trial.

Review of Literature

Fats are integral to our diet, supplying not only caloric density but also essential fatty acids. Fats improve the palatability, give satiety value and increases absorption of fat-soluble vitamins. Fat intake, is influenced by social, cultural, economic & geographic factors. (Rukmini C., Raghuram T.C . *Journal of American College of Nutrition.* 1991,10:593-601)

Because of the dynamic relationship between changes in people's diet, rapid socio-economic development, decreasing trend in infectious disease due to better health care and consequently longer life expectancy, chronic degenerative diseases are increasing even in developing countries. (Bamji. M.S., Prahlad Rao N, Reddy Vinodini, *Textbook of Human Nutrition.* 1998)

CAD is the leading cause of death in developed countries. The disorders that lead to the process of atherosclerosis and thrombosis (coronary artery disease, stroke and peripheral vascular diseases) are generally associated with affluence and lifestyle such as faulty dietary habits, high levels of stress, smoking, alcoholism, tobacco chewing and sedentary living. Available evidence indicates that CAD, hypertension, diabetes and obesity are increasing in the population particularly in urban areas. (Bamji. M.S., Prahlad Rao N, Reddy Vinodini, *Textbook of Human Nutrition.* 1998,3,15)

AHA presents the guidelines for reducing the risk of cardiovascular disease by dietary and other lifestyle practices. The principle underlying the AHA guideline is that there are dietary and other lifestyle practices that all individuals can safely follow throughout their

lifespan as a foundation for achieving and maintaining cardiovascular and overall health. (*AHA Dietary Guidelines*. Circulation 2000;102:2284-2299)

Dietary fats have been shown to have distinct influence on cardio-vascular diseases. A relationship between CAD, elevated concentration of serum cholesterol and dietary cholesterol has been established and recent studies have shown that the risk of CAD can be reduced by lowering serum cholesterol concentrations. The scientific positioning of AHA is that high total and LDL cholesterol levels are strongly related to CAD risk. Hence dietary manipulation is known to play an important role in management of hyperlipidemia. Since the quality of dietary fat is an important determinant in the regulation of plasma cholesterol level, considerable attention has been focused on intake of dietary fat. (Seetharamaih G.S, Krishnakantha T.P and Chandrasekhara N. *Journal of Nutritional Science Vitaminol* 1990, 36:291-297, Qureshi A. et. at. *American Journal of Clinical Nutrition.* 1991:53: 1021S-6S, Deckhere E.A.M, Kornel O. *Nutrition Reviews.* 1996, 54:S120-S126, *American Heart Association Scientific statement.* Circulation, 1998;97:1876-1887, *AHA Dietary Guidelines.* Circulation 2000;102:2284-2299)

Dietary fats are derived from both plant and animal sources and are classified as "visible" and "invisible" types. Invisible fats are present as an integral component of different foods, whereas fats that are used for cooking (vegetable oils, vanaspati, & animal oils such as butter and ghee) are termed as "visible" fats. Oils are part of traditional diets all over the world. (*Nutritional Institute of Nutrition*, ICMR, Hyderabad, 1999)

Depending on the predominant fatty acids (chemical components of fat) present fats are grouped as monounsaturated, polyunsaturated and saturated. There is consensus that the fatty acids balance in the diet is important (Deckhere E.A.M, Kornel O. *Nutrition Reviews*. 1996, 54:S120-S126, *Nutritional Institute of Nutrition*, ICMR, Hyderabad, 1999). Over years there is extensive evidence to corroborate the hypocholesteremic effect of vegetable oils which are rich in polyunsaturated fatty acids (PUFA) particularly linoleic acid and linolenic acids which are present only in plant foods. Much emphasis has also being given to the hypolipidemic effect of the monounsaturated fatly acids. (MUFA) (*Nutritional Institute of Nutrition*, ICMR, Hyderabad. Raghuram T.C, *Nutrition composition and Hypolipidemic effects of Rice Bran Oil. AHA Dietary Guidelines*. Circulation 2000;102:2284-2299)

Nowadays, there is consensus that fatty acids balance in the diet is important (Deckhere E.A.M, Kornel O. *Nutrition Reviews*. 1996, 54:S120-S126, *Nutritional Institute of Nutrition*, ICMR, Hyderabad,) more so because, of late, intake of oils highly rich in PUFA, has been reported to be carcinogenic. (Bagga D et al. *Nutrition and Cancer*. 2002; 42(2):180-185, McEntee MF, Whelan J. *Biomedical Pharmacother*. 2002; 56(8): 380-387, Marnett LJ. *Toxicology*. 2002 Dec 27; 181-182:219-222 , Wirfalt E et al. *Cancer Causes Control*. 2002; 13(10):883-893)

There have been numerous studies in humans and animals that have demonstrated that oil containing saturated fatty acids (SFA) raise total cholesterol and in particular LDL-cholesterol (Wilson T.A et al. *Journal of American College of Nutrition*. 2000, 19:601-607). Therefore, saturated fats are atherogenic and mono and poly-unsaturated fats are anti-atherogenic. *(Nutritional Institute of Nutrition,* ICMR, Hyderabad, 1999)

Public health programs for prevention of CAD in developed countries recommend changes in the dietary habits and patterns of various components of diet especially dietary fat. Recently a reduction in the dietary intake of total fat, particularly animal fat with a limited amount of SFA and increase in MUFA/PUFA has been suggested (Rukmini C., Raghuram T.C. *Journal of American College of Nutrition.* 1991,10:593-601). The dietary guidelines of AHA, revised in 2001, in general recommends a diet that contains ≤ 30% of energy as fat, ≤ 10% energy as saturated fatty acids, 8-10% energy as PUFA and < 300 mg cholesterol per day (*AHA Dietary Guidelines.* Circulation 2000;102:2284-2299). According to the Recommended Dietary Allowances for Indians, the total fat (invisible + visible) in the diet should provide 15-30% total calories. Studies, suggest that an intake of fat energy at 30 % or more of total calories is undesirable. Some 10 – 15 % of this can come from invisible fat, visible fat intake should be kept below 15 %. None of the edible oils has this optimum composition; hence use of more than one source of fat/oil has the added advantage of providing a variety of minor components in the diet. (Rukmini C., Raghuram T.C. *Journal of American College of Nutrition.* 1991,10:593-601, *Nutritional Institute of Nutrition*, ICMR, Hyderabad, 1999) As per the recommendation of NIN, amongst others, a blend of Rice bran oil with Sunflower oil is a healthy option. (Ghafoorunissa. *Ind J Med Res* 1998; 108 191-202)

Besides the fatty acid composition, it is recognized that minor components present in dietary fats such as: tocopherols, tocotrienols, sterols, etc, also play an important role in the regulation of plasma cholesterol levels. Since most of these components are antioxidants, they prevent fats from going rancid. (*Nutritional Institute of Nutrition*, ICMR, Hyderabad)

In this regard among the vegetable oils Rice Bran oil has been receiving considerable attention in recent years in view of its beneficial effects (*AHA Dietary Guidelines*. Circulation 2000;102:2284-2299. Raghuram T.C, *Nutrition composition and Hypolipidemic effects of Rice Bran Oil*.)

RBO contains oleic acid (38.4%), linoleic acid (34.4%) and linolenic acid (2.2 %) as unsaturated fatty acids and palmitic (21.5%) and stearic (2.9%) acids as saturated fatty acids. The unsaponifiable fraction (4.2%) has total tocopherols (81.3 mg%), γ-oryzanol (1.6%) and squalene (320 mg%) (Rukmini C., Raghuram T.C. *Journal of American College of Nutrition.* 1991,10:593-601).

A study of various rice bran oil brands available in the market showed that the saturated fatty acid content varies from 21.9 % to 27.8%, MUFA content varies from 41.3% to 49.9% and PUFA content varies from 24.4% to 36.6%.

Tocopherols and tocotrienols which are present in high concentration provides oxidative stability to RBO and improve its shelf- life. (*AHA Dietary Guidelines*. Circulation 2000;102:2284-2299. Raghuram T.C, *Nutrition composition and Hypolipidemic effects of Rice Bran Oil*) Oryzanol is a mixture of ferulic acid, esters of triterpene alcohols, such as cyclo artenol (CA 106 mg%) and 24 -- methylene cycloartenol (494 mg %). It is reported that cycloartenol, a triterpene alcohol, present in high amounts in RBO, is absorbed and accumulated in liver. Since its chemical structure is similar to cholesterol, it competes for the cholesterol binding sites causing greater metabolism and excretion of cholesterol as bile salts and pigments. (Rukmini C., Raghuram T.C. *Journal of American College of Nutrition.*

1991,10:593-601. *AHA Dietary Guidelines*. Circulation 2000;102:2284-2299. Raghuram T.C, *Nutrition composition and Hypolipidemic effects of Rice Bran Oi.*)

Oryzanol has been investigated for its hypolipidemic activity by several workers in rats, in mice and in human subjects. (Raghuram T.C, Brahmaji Rao U, Rukmini.C, *Nutrition Reports International*. 1998; 39:889-895., Seetharamaih G.S, Krishnakantha T.P and Chandrasekhara N. *Journal of Nutritional Science Vitaminol* 1990, 36:291-297., Sharma R.D and Rukmini C. *Rice Bran Oil and Hypocholesterolemia in Rats*, 1986; 21: 715 – 718. Vissers M.N et al. *American Journal of Clinical Nutrition*. 2000; 72:1510 – 1515)

The efficacy of oryzanol in counteracting fructose-induced hypertriglycerdemia was reported by Setharamaiah and Chandrashekara. In another study, by them they have reported that oryzanol fed along with 1% cholesterol diet significantly inhibited platelet aggregation in rats, induced by ADP and totally inhibited aggregation induced by collagen. (Seetharamaih G.S, Krishnakantha T.P and Chandrasekhara N. *Journal of Nutritional Science Vitaminol* 1990, 36:291-297.)

R.J Nicolosi has indicated that Rice Bran oil contains unsaponifiable component, which lowers LDL level by mechanisms, which appears to be not dependent on the fatty acid composition of the diet. These unsaponifiables occur in highest concentration in RBO. He also mentioned that RBO contains very potent antioxidants which reduce the susceptibility of LDL to oxidation. Thus, these preliminary studies suggest that RBO and certain of its components have potentially interesting health benefits. (Nicolisi R.J. *Department of Clinical Sciences*, Universities of Lowell)

In a study carried out by Sharma and Rukmini on rats (Sharma R.D and Rukmini C. *Rice Bran Oil and Hypocholesterolemia in Rats*, 1986; 21: 715 – 718), they showed that rats fed rice bran oil at 10% level for a period of eight weeks showed significantly lower levels of TC, LDL and VLDL, both on cholesterol containing and cholesterol free-diets. HDL cholesterol was increased & triglyceride showed a decrease. Liver cholesterol and liver triglycerides were also reduced. Fecal excretion of neutral sterols and bile acids was increased after ingestion of RBO. Other investigators have also reported that the addition of oryzanol to an RBO containing diet potentiates the hypolesterolemic action of RBO in rats. (Rukmini C., Raghuram T.C . *Journal of American College of Nutrition.* 1991,10:593-601)

The hypolipidemic action of dietary RBO was also investigated in human subjects by Raghuram et.al. Twelve subjects either with high serum cholesterol or high triglycerides were advised to use RBO in place of other cooking oils, which they were using earlier. There was a significant reduction in serum cholesterol and triglyceride levels after 15 and 30 days after the use of RBO in the diet. There was another group of nine control subjects where there were no changes in the serum cholesterol and triglyceride levels. They have concluded that RBO could be considered as a edible oil of preference for patients with abnormalities of lipid metabolism (Raghuram T.C, Brahmaji Rao U, Rukmini.C, *Nutrition Reports International.* 1998; 39:889-895.) Vissers et.al. in years 2000, have also reported that rice bran oil lowered serum total cholesterol by 5 % and LDL cholesterol by 9% in normolipemic humans. (Vissers M.N et al. *American Journal of Clinical Nutrition.* 2000; 72:1510 – 1515)

Rice is a major cereal consumed in India. Rice Bran is a by –product of the rice milling industry. Bran is a part between paddy husk and endosperm and it consists of

15 – 20% oil. India is the second largest paddy producer in world. Rice Bran oil, is being used as an edible oil in Japan since centuries. China, Korea and other countries also use RBO as an edible oil. Edible grade RBO obtained by improved technology was systematically evaluated for its chemical, nutritional and toxicological properties at NIN by C. Rukmini and the multigenerational toxicological studies established the safety of RBO for human consumption. (*AHA Dietary Guidelines*. Circulation 2000;102:2284-2299. Raghuram T.C, *Nutrition composition and Hypolipidemic effects of Rice Bran Oil*, Rukmini C. Chemical, *Food chemistry*, 1988, 30:257 – 268)

Even the rice bran fiber is effective in reducing the Serum total cholesterol, LDL cholesterol, apolipoprotein B and triglyceride levels. Also, it reduces the glycoselated hemoglobin as well as the fasting glucose levels in Type I and Type II diabetics. (Qureshi AA et al. *Journal of Nutritional Biochemistry*. 2002; 13(3): 175-187).

In view of the beneficial effects of RBO and the recommendation by NIN of using a combination of oils, Ricebran and Sunflower being one of them the present study was undertaken to study the effect of the blend (Ricebran oil and Sunflower oil in 80:20 ratio and 5000 ppm Oryzanol) on the lipid profile (TC, LDL, HDL, VLDL & Tgl) of hyperlipidemic as well as normolipidemic subjects.

The reasons of choosing a blend rather than Ricebran oil alone were the following:

1. The NIN recommendation is to consume more than one oil rather than a single oil alone.

2. Given that the 100% RBO varies a lot in it's fatty acid profile, depending on the season and the geographical location, a blend offers an opportunity of consistently providing a fatty acid profile closer to the AHA recommendation.

Methodology

Sample Details:

The control product used was sunflower and groundnut, depending on the usual consumption pattern of the subjects. These were branded oils, purchased from the local market. For the test group, the oil used was a blend of ricebran oil and sunflower oil provided by AgroTech Foods Ltd, Secunderabad.

No. of subjects:

Hyperlipidemic – 32 (1A1 – 16 & 1A2 - 16)

Normolipidemic – 16 (1B)

Keeping in mind the objects of study: The 32+16 subjects were randomly selected. Total no of subjects screened were 232 as per the protocol design & those who met the inclusion / exclusion criteria of the study were enrolled for the study conducted at Apollo hospitals, Jubilee Hills, Hyderabad. Beyond the 32 + 16 subjects selected for this study, the balances were selected for other studies, not relevant to this study.

Study Design:

Single – blind switching replicating study in healthy subjects who received test product according to a predetermined randomized order allocation. Test product refers to RBO + SFO (80:20 ratio with 5000 ppm of Oryzanol) blend & the control product is the subject's usual cooking oil.

Study Schematic:

Table 1: Study Schematic

<table>
<tr><td>Group</td><td colspan="4">1 A (n=32)</td><td colspan="2">1B(n=16)</td></tr>
<tr><td rowspan="2">LIPID STATUS</td><td colspan="2">1A1 (n=16)</td><td colspan="2">1A2 (n=16)</td><td colspan="2" rowspan="2">NORMOLIPIDEMIC</td></tr>
<tr><td colspan="2">HYPERLIPIDEMIC</td><td colspan="2">HYPERLIPIDEMIC</td></tr>
<tr><td rowspan="3">OIL GIVEN
Day 0 – Day 30</td><td colspan="2" rowspan="3">Test (Blend)</td><td>(n=8)</td><td>(n=8)</td><td colspan="2" rowspan="3">Test (Blend)</td></tr>
<tr><td>Ctrl</td><td>Ctrl</td></tr>
<tr><td>(SFO)</td><td>(GNO)</td></tr>
<tr><td rowspan="3">OIL GIVEN
Day 31- day 60</td><td>(n=8)</td><td>(n=8)</td><td colspan="2" rowspan="3">Test (blend)</td><td>(n=8)</td><td>(n=8)</td></tr>
<tr><td>ctrl</td><td>ctrl</td><td>Ctrl</td><td>Ctrl</td></tr>
<tr><td>(SFO)</td><td>(GNO)</td><td>(SFO)</td><td>(GNO)</td></tr>
</table>

Inclusion Criteria:

Subjects, which met the following criteria, were enrolled in the study.

- Males or Females between the ages of 25 – 60 years inclusive.
- Had high level of blood lipids TC>200 mg/dl, or Tgl > 150 mg/dl (for groups 1A) and normal level of cholesterol TC:180-200 mg /dl and Tgl <150 mg /dl (for group 1B) at the time of pre-entry evaluation.
- Were consuming at least 30 gm of visible fat on an average daily basis.
- Have given written consent to participate in the study.

Exclusion Criteria:

Subjects with the following conditions were not enrolled in the study.

- Those suffering from any serious or debilitating medical condition
- Females with pregnancy

- On treatment with any medication which would alter or influence blood cholesterol / Triglyceride levels.
- Current drug or alcohol abuse as determined by medical history.
- History of diabetes
- Subjects eating out frequently, due to outstation travel etc.
- Those who were consuming RBO oil on a regular basis.
- Consumes Vanaspati or Ghee predominantly as a cooking/frying medium.

Administration

A. Ethics Committee

The protocol was explained and submitted to Central Ethics Committee of Apollo Hospitals, Jubilee Hills and the approval for conducting the trial was obtained.

B. Subject's Consent

Written Informed consent was obtained from all the subjects before including them in the study. All the elements of informed consent was explained to each participant in clear, concise layman's language, stating the purpose and expectations of the study. The participant was also explained about the no. of visits to be made during the course of the study.

Study Implementation

Screening Stage:

Subjects who consumed sunflower and groundnut oil on a regular basis were short listed, while subjects consuming Rice bran oil were excluded. The Preliminary Health Scan (PHS) questionnaire was then administered to record all the Lifestyle attributes. The information collected included cardiac risk factors, exercise & fitness, dietary habits, stress, dependencies, kind and quantity of oil consumed at home, etc. Based on PHS information the subjects who were on treatment with any medication which could influence lipid profile or who were consuming alcohol daily or who had history of diabetes were excluded from the study.

The short listed subjects were then screened for Fasting blood sugar and complete lipid profile. Sampling and analysis methods are explained later. The subjects who were not diabetic but showed high levels of sugar were tested for GTT and when confirmed for diabetes were excluded from the study.

The short-listed candidates who matched protocol criteria of hyperlipidemia were randomly allocated. 32 number were taken in group A (16 in 1A1 and 16 in 1A2) & 16 subjects whose cholesterol level was not more than 200 mg/dl but was between 180 – 200 mg/dl were allocated to normolipidemic group i.e., group B. All the 48 subjects were then called for baseline or Day 0 visit.

Visit Day – 0:

Before including subjects in the study Cardiologist's opinion & complete medical history was taken, Physical examination and ECG was done. Subjects who were found to be fit by cardiologist with normal ECG and therefore not requiring immediate medical attention were finally included in the study.

On the day – 0 the oil to be consumed by subjects were distributed. The quantity distributed was sufficient enough for the entire family as per their regular intake. At day – 0 visit the following type of oil were given to different groups.

Table 2: Oil Given at Day 0 Visit.

Groups	1A1	1A2	1B
n =	16	16	16
Oil Given	Blend	SFO/GNO	Blend

Subjects then were asked to come after 15 days.

Visit day – 15:

On the day 15 following procedures were carried out as per protocol guidelines.

1. Blood sample was drawn for lipid profile analysis i.e., T.C, LDL, HDL, VLDL and Tgl.
2. 24 Hr dietary recall was taken.
3. The PHS questionnaire was filled in to record lifestyle attributes.

4. Patients were also asked for any concomitant medication or treatment taken or any adverse event happened to them during the course of study.
5. The type and quantity of oil and snacks given to subjects in all the groups was same as on day – 0 visit.

Visit Day – 30:

For the subjects in group A&B, on the day 30th visit i.e. after 1 month was crossover of oil consumed by them. The group that was consuming test (new blend) oil was switched to the control oil (Regular cooking oil i.e. SFO or GNO) and the group consuming regular oil was put on test oil (new blend).

Table 3: Oil Given at Day 30 Visit

Groups	1A1	1A2	1B
n =	16	16	16
Oil Given	SFO/GNO	Blend	SFO/GNO

Other procedures carried out at Day – 30 visit were –

1. Cardiologist consultation for all the subjects
2. Blood sample was taken for lipid profile analysis: TC, LDL, HDL, VLDL & Tgl.
3. 24 hr dietary Recall was taken.
4. The PHS questionnaire was administered to monitor any deviation in their lifestyle attributes.
5. Patients were also asked for any concomitant medication or treatment taken or any adverse event occurred during the course of study.

Visit day – 45:

Distribution pattern of oil and snacks for group A and B was same as on Day 30 visit. Other procedures carried out were.

1. Blood sample was taken for lipid profile analysis: TC, LDL, HDL, VLDL & Tgl.
2. 24 hr dietary Recall was taken.
3. The PHS questionnaire was administered to monitor any deviation in their lifestyle attributes.
4. Patients were also asked for any concomitant medication or treatment taken or any adverse event occurred during the course of study.

Visit Day – 60:

This was the last visit for group A and B. Blood sample was taken for lipid profile analysis to check the lipid status at the end of the study. Other procedures carried out were

1. 24 hr. dietary Recall was taken.
2. The PHS questionnaire was administered to monitor any deviation in their lifestyle attributes.
3. Patients were also asked for any concomitant medication or treatment taken or any adverse event occurred during the course of study.

At the end of the study again the cardiologist's consultation was done for all the subjects in-group A and B which included physical examination and review of lipid profile results of all the visits. Appropriate counseling was also done in Lifestyle by PHS Counsellor.

Biochemical Analysis Methods:

Following methods were used for analysis.

a. *Cholesterol Automated Method:* The CHOL method used is based on the principle first described by Stastman, later adapted by other co-workers.
b. *Triglycerides Automated Method:* The TRIG method is based on an enzymatic procedure in which a combination of enzymes is employed for a kinetic bichromatic measurement of serum triglycerides.
c. *HDL Automated Method:* The AHDL method is a homogenous method for directly measuring HDL levels without the need for off-line pre-treatment of centrifuges steps.
d. *LDL (Calculated):* Using Friedwald's formula
e. *Total Cholesterol/HDL ratio:* Calculated.

Sample Collection for Biochemical Analysis:

A minimum of 15 ml of venous blood sample was collected from subject at each visit. Blood sample was drawn from the forearm. 7.5 ml of plasma was used for the analysis. The balance plasma was stored (at -20^{0} C) for any future analysis if required.

PHS Questionnaire: Appendix A.

Results

Out of the 32 subjects enrolled in the hyperlipidemic group, 3 subjects did not use the test oil for 1 week continuously and hence were dropped from the study. The results of the 29 subjects are given below:

Table 4: Effect of blend oil on Hyperlipidemic group

	Sample Size (N)	MEAN	S.E	SIG.
TC 0	29	218	5.99	
TC 30	- do-	202.1*	6.71	.002
TC 60	- do-	217.7	5.72	.472
LDL 0	- do-	141.7	6.70	
LDL 30	- do-	131.9*	5.57	.042
LDL 60	- do-	142.3	5.50	.443
HDL 0	- do-	43.5	1.32	
HDL 30	- do-	42.4	1.59	.121
HDL 60	- do-	44.6	1.51	.181
Tgl 0	- do-	163.9	14.81	
Tgl 30	- do-	139.2*	10.94	.012
Tgl 60	- do-	154.8	12.58	.203
VLDL 0	- do-	32.8	2.96	
VLDL 30	- do-	27.9*	2.20	.012
VLDL 60	- do-	30.9	2.52	.186

In hyperlipidemic group, it was observed that after using the new blend oil for 30 days, there was statistically significant ($P<0.05$) reduction in mean cholesterol values from 218 to 202. However, when the group was switched to their regular oils (SFO/GNO) for next 30 days, the total cholesterol values went up and it reached close to the baseline value. This can be seen in the results wherein there is no significant difference between the baseline Total

cholesterol value and the cholesterol values after consumption of the regular oil. This clearly shows the efficiency of the new blend oil in reducing the total blood cholesterol.

This hyperlipidemic group also showed a statistically significant reduction ($P<0.05$) in total triglycerides from the initial value of 163.9 to a final value of 139.2, after using the new blend oil for 30 days. And when the subjects switched to their regular oil, the total triglycerides increased and almost reached the baseline values. The difference in the level of triglycerides from day 30 to day 60 is non-significant, showing the similar effect which was seen in total cholesterol.

Similarly LDL cholesterol and VLDL cholesterol also showed statistically significant reduction ($P<0.05$) after using blend oil for 30 days.

Table 5: Effect of blend oil on Normolipidemic group

	Sample Size(N)	Mean	S.E	Sig (2-tailed)
TC 0	16	191.0	1.27	
TC 30	16	183.2*	3.48	.009
TC 60	16	183.4	3.48	.067
LDL 0	16	123.2	2.17	
LDL 30	16	117.2*	3.03	.046
LDL 60	16	117.1	5.70	0144
HDL 0	16	46.3	1.8	
HDL 30	16	46.0	1.83	.445
HDL 60	16	44.5	1.96	.121
Tgl 0	16	107.4	7.71	
Tgl 30	16	99.6	7.92	.070
Tgl 60	16	109.1	7.89	.413
VLDL 0	16	21.4	1.54	
VLDL 30	16	19.9	1.56	.081
VLDL 60	16	21.8	1.59	.405

The above data indicates that when normolipidemic group was put on the new blend oil for 30 days, it showed statistically significant reduction ($P<0.05$) in total cholesterol from 191 at day 0 to 183.2 at day-30 and LDL cholesterol from 123.2 to 117.2. The other parameters like , VLDL cholesterol and total triglycerides also showed reduction but these were not statistically significant. However when the group was switched to their regular oil (SFO/GNO) there was increasing trend in the lipid profile and it was not statistically different from the baseline value.

Table 6: Lifestyle Scores

Group	Lifestyle Score At	N Statistic	Mean	S.E	Sig (2-tailed)
1A1	L0	16	63.3125	1.7023	
	L30	16	63.0625	2.0665	0.9209
	L60	16	66.3125	1.474	0.0790
1A2	L0	16	57.875	2.3539	
	L30	16	60.0625	2.4605	0.1655
	L60	16	60.5625	2.0816	0.1020
1B	L0	16	64.0625	1.8763	
	L30	16	67.125	2.2246	0.0609
	L60	16	63.6875	2.3536	0.8615

Lifestyle scores for all the subjects was calculated at every visit in all the groups. Mean lifestyle scores of groups at 30 days and 60 days from baseline indicated that their was not statistically significant difference in the lifestyle scores. This indicates that Lifestyle factors including diet, exercise, stress, smoking, alcohol intake etc. in all the groups was same during the course of the study.

Conclusions

Result of the study indicated that in hyperlipidemic subjects, consumption of the test blend (Blend of Ricebran and Sunflower in 80:20 ratio with minimum 5000 ppm of Oryzanol) for 30 days showed reduction in the lipid profile parameters at day 30. Total cholesterol, LDL cholesterol, VLDL cholesterol and Total Triglycerides showed statistically significant reduction.There was no significant cnange in HDL cholesterol levels. The hyperlipidemic group when switched to their regular oil for next 30 days showed an increasing trend in lipid profile at day 60; and the values at day 60 were not statistically different from day 0 (baseline) values. This indicates that the new blend oil was effective in reducing the lipid status of hyperlidemic subjects.

In normolipidemic subjects, consumption of the test blend oil for 30 days also showed reduction in the lipid profile. Total cholesterol and LDL cholesterol showed statistically significant reduction in this group at day 30. Other parameters like VLDL cholesterol and total triglycerides also showed reduction but were not statistically significant. To study whether reduction in lipid profile was due to oil used and not due to change in any lifestyle parameter, lifestyle of subjects was assessed at all the visits. Lifestyle scores were then subjected to statistical analysis. It was indicated that there was no difference in the lifestyle of the subjects at day 30 and day 60.

It can be therefore concluded that blend oil was effective in reducing lipid profile parameters and that change could not be attributed to other factors like dietary habits, exercise, stress etc. because lifestyle factors were more or less constant during the course the study.

Annexure

Preliminary Health Scan Questionnaire

This Scan may be used by a physician or health practitioner for assessing and advising on Cardiac, Cancer and AIDS risk, Fitness and Exercise, Nutrition, Stress, Dependency, etc. FOR PERSONS OF 18 YEARS AND ABOVE.

IDENTITY ENTRY

REGISTRATION NO: .....
NAME: .....
ADDRESS: .....
.....
HEIGHT (Ft/Ins): ..... Cms.....
GENDER (M/F): ..... WEIGHT: .....(Kgs)
TELEPHONE NO: ..... AGE: ..... (Years)
EUROPEAN DESCENT (Y/N): .....
DATE OF CHECK UP: .....
LANGUAGES SPOKEN: (1) ..... (2) .....

FOR VISUALLY IMPAIRED PERSONS, TICK HERE ☐

TESTS

The following test results, if indicated, will give a more precise prescription. Please undergo as many of these tests as possible. These tests are contained in the questionnaire and should preferably be filled in the presence of a health professional.

QUESTION NO.		TESTS
Q.2	—	ECG
	—	TMT
	—	Echo Cardiogram
Q.3	—	Blood Pressure
Q.4	—	Blood Sugar - Fasting - Random
Q.5	—	Total Cholesterol - HDL - VDL - VLDL - Triglycerides

Q.7	—	Hemoglobin
Q.11	—	Pulse Rate
Q.12	—	PFT
	—	Breath Test
Q.13	—	Pinch Test
	—	Skin Fold Caliper
Q.14	—	Sit-ups
Q.15	—	Toe Touch
Q.56	—	PAP Smear
		Mammography

QUESTIONNAIRE

		NO	Don't know	YES
1)	Has any member of your close family (parents, grandparents, siblings) had of the following conditions before the age of 60 years?	☐	☐	☐
	Heart Attack ☐ High Blood Pressure ☐ Diabetes ☐ Hyper Cholesterol ☐			
2)	Do you have a Coronary Heart Disease? (Angina, Heart Attack, Angioplasty, Bypass)	☐	☐	☐

If aware, please indicate results of the following:

ECG	normal ☐	abnormal ☐
TMT	normal ☐	abnormal ☐
Echocardiogram	normal ☐	abnormal ☐

Comments, if any .....

.....

.....

3) Do you have high Blood Pressure? ☐ ☐ ☐

If aware, indicate your Blood Pressure:.....

4) Do you have high Blood Sugar levels? ☐ ☐ ☐

If aware, please indicate your Blood Sugar levels:
Fasting:.....2 hrs Post Prandial.....
Random: .....2 hrs Post Glucose.....

5) Do you have high Cholesterol / Triglycerides? ☐ ☐ ☐

If aware, please give details:

Total Cholesterol	: .....
H.D.L	: .....
L.D.L	: .....
V.L.D.L	: .....
Triglycerides	: .....

NO YES

6) In the last twelve months, have you had any major accident/ surgery / hospitalization? ☐ ☐
Please specify .....

Please mention the major medical/surgical procedure(s),
if any, that you have undergone in the past
.....

7) Do you suffer from Anemia? ☐ ☐

If aware, indicate your hemoglobin % .....

8) Are you frequently under medication? ☐ ☐

If taking any medication, please tick the reason:

High Blood Pressure	☐
Diabetes	☐
Hyper Cholesterol	☐
Cardiac Ailment	☐
Respiratory problem	☐

Other problems .....
.....

9) Is it difficult for you to exercise partially or totally due to any medical reason? ☐ ☐

Please tick if any of the following apply to you:
Orthopedic Impairment ☐
Amputation of limb(s) ☐
Polio ☐
Muscular dysfunction ☐
Paralysis / Stroke ☐
Any other reason .....
.....

10) Do you exercise for at least half an hour three days a week? (If your occupation is manual/physical, tick Yes) ☐ ☐

11) Is your resting pulse rate below 72 beats per minute measured while sitting? ☐ ☐

Please indicate your Pulse Rate: ..... beats/min

NO YES

12) Take a deep breath. Can you hold your breath for 45 seconds? ☐ ☐
Indicate maximum duration: ..... secs.

If you have undergone a Pulmonary Function Test, please tick results:
PFT normal ☐ abnormal ☐

13) With your forefinger and thumb, pinch a roll of flesh at your waist (above the hip bone).
If male, is it more than 2.5 cm. thick?
If female, is it more than 3 cm. thick? ☐ ☐

If you have undergone a skin fold caliper test, please indicate measurement: ..... mm

14) Can you complete continuous sit ups as per your age chart below? ☐ ☐
Indicate maximum done: .....

(Do not attempt more than 30 repetitions)

Age	Male	Female
Upto 30 yrs.	20	10
Upto 40 yrs.	15	7
Upto 50 yrs.	10	5
Above 50 yrs.	5	3

15) Can you touch your toes without bending your knees? ☐ ☐

PINCH TEST - Q.13

SIT UP TEST - Q.14

TOE TOUCH - Q.15

16) Can you identify foods that are high in protein, carbohydrate, fat and fiber? ☐ ☐

17) Are you a vegetarian? ☐ ☐

18) Do you eat raw uncooked vegetables/fruit daily? ☐ ☐

19) Do you eat beans, dal, lentils or gram almost daily? ☐ ☐

OR

Do you eat meat more than three days a week?

NO YES

20) Do you eat any of the following foods more than twice a week (whole cream milk, butter, cheese, ghee, dalda, margarine, etc.) OR eggs and red meats (mutton, beef, pork, organ meat)? ☐ ☐

1) Do you eat puddings, cakes, mithai, sweets, chocolates, ice-cream, etc. frequently, or take more than 4 teaspoons of sugar daily? ☐ ☐

2) Do you drink more than six glasses of water daily? ☐ ☐

23) Do you use excess salt in your food, or eat salted snacks (nuts, wafers, biscuits, etc)? ☐ ☐

24) Do you eat out twice a week or more at hotels; restaurants, commercial food centers, etc, or frequently consume commercially-prepared foods like coffee, jam, pickles, sauces, bottled drinks, tinned & packaged or smoked foods, etc? ☐ ☐

25) Were / are either of your parents fat, or were you a fat child up to the age of 18 years? ☐ ☐

26) Do you generally eat a heavy dinner, snack between meals or eat your meal at irregular timings?

Do you experience any of the following frequently? (Tick Yes or No, and if aware indicate reasons):

	NO	YES
27) Headaches (migraine, tension, ...)	☐	☐
28) Respiratory ailments (asthma, hay fever, bronchitis, ...)	☐	☐
29) Digestive problems (ulcers, irritable bowel, gassiness, acidity, ...)	☐	☐
30) Musculoskeletal problems (arthritis, spondylosis, back pain, ...)	☐	☐
31) Skin problems (psoraisis, eczema, rash, ...)	☐	☐
32) Immune disorders (allergies, infections, ...)	☐	☐
33) Insomnia (initiating, maintaining or going back to sleep .....)	☐	☐

(Tick Yes or No, and if aware indicate reasons):

	NO	YES
34) Do you have friends/relatives whom you can turn to for support and help?	☐	☐

35) Are you emotionally/physically compatible with your family members? ☐ ☐

36) Does your occupation give you adequate returns and hold promise for the future? ☐ ☐

37) Does your occupation result in neglecting your home, recreation and social life? ☐ ☐

38) Are you frequently time-pressured and impatient (hate waiting in line, at traffic lights, interrupt conversations, etc)? ☐ ☐

39) Are you basically competitive (compete with others, feel compelled to win, hate to lose)? ☐ ☐

40) Do you prefer to avoid conflicts and avoid discussing any differences at all costs? ☐ ☐

41) Do you find it difficult to give your needs as much importance as others' needs? ☐ ☐

42) When faced with problems are you able to plan for solutions ☐ ☐

43) Are you able to evaluate and accept the merit of ideas different from your own and are able to delegate? ☐ ☐

If you were faced with any of the situations mentioned in Q.41-44 during the last 12 months, or are currently having these problems, tick Yes or No

44) Death of a significant person ☐ ☐

45) Major illness of a significant person ☐ ☐

46) Separation from family or divorce ☐ ☐

(Tick Yes or No, and if aware indicate reasons):	NO	YES
47) Are you currently faced with any of the problems mentioned below	☐	☐

Please tick your problem area(s), if any:

- Constant & daily hassles (frequent irritants at home / work, etc.) ☐
- Major family problem (marriage, children, residence, in-laws, etc.) ☐
- Major health problem ☐
- Major financial problem ☐
- Major career problem (change, termination, retirement, etc.) ☐
- Any legal problem ☐
- Other problems not mentioned above ☐

	Never	Occasionally	Daily
48) Do you smoke?	☐	☐	☐
49) Do you take alcohol?	☐	☐	☐
50) Do you use paan, paan bahar, supari, tobacco, etc?	☐	☐	☐

	NO	YES
51) Are you a passive smoker i.e. exposed to smokers for long hours daily?	☐	☐
*52) Have you been smoking 10 cigarettes or more daily for the last 7 years or more?	☐	☐
*53) Have you been drinking more than 2 pegs of alcohol or its equivalent almost every day since the last 10 years or more?	☐	☐
*54) Have you been chewing paan, paan bahar, supari, tobacco, etc. almost daily for the last 7 years or more?	☐	☐
55) Do you use narcotic drugs (marijuana, opium, hashish, heroin, etc)?	☐	☐

(Answer NO only if given up or abstained for the last two years)*

56) If fair skinned, do you frequently expose your skin to sunlight for long hours and suffer sunburn? ☐ ☐

57) During the last seven years, have you been frequently exposed to industrial chemicals, fumes, dust, radiation, gasoline, paint, etc? ☐ ☐
NO YES

58) Do you currently have Cancer, or have you had cancer in the past? ☐ ☐

If yes, please give details:
Current: .....
Past: .....

59) Have you undergone a Cancer check-up during the last one year? ☐ ☐

For ladies who have undergone a PAP Smear, please tick results here:
PAP Smear normal ☐ abnormal ☐
.....
Mammography normal ☐ abnormal ☐
.....

60) Has any member of your close family (parents, grandparents, siblings) had cancer? ☐ ☐

Female specific Cancers (breast, uterus, ovaries) ☐
Male specific Cancers (prostate, etc.) ☐
General Cancers (lung, colon, skin, etc.) ☐

61) Do you have difficulty in hearing? ☐ ☐

If deaf, tick here ☐

62) Do you wear spectacles or contact lenses for improving your vision? ☐ ☐

63) Do you have recurrent problems with any of the following? ☐ ☐

If so, tick box below:

Ears	☐
Nose	☐
Throat	☐
Gums & Teeth	☐

NO YES

64) In order to preserve confidentiality, details on HIV/AIDS appear separately in your prescription. However, if you wish you may indicate details here: ☐ ☐

HIV test results:

Negative	☐
Positive	☐
Not aware	☐

65) Do you always observe the basic rules of home safety (i.e., protection from fire hazards, safe usage of gas & electricity, safety railings in balconies & stairways) and other such measures? ☐ ☐

66) In your occupation/work, do you adhere to all the safety regulations and stipulations? ☐ ☐

67) While commuting, do you observe relevant safety regulations (seat belts, helmets, speed limits, traffic regulations, etc)? ☐ ☐

68) Do you frequently engage in contact sports, motor sports, or adventure activities including water sports, aero sports, mountain climbing, etc? ☐ ☐

FOR LADIES ONLY (Tick the relevant box)	NO	YES
69) Are you pregnant?	☐	☐
70) Do you have your own biological offspring?	☐	☐
71) Are you currently breastfeeding?	☐	☐
72) Do you frequently suffer discomfort during Menstruation?	☐	☐
73) Have you reached menopause?	☐	☐
74) As a women, do you feel subjected to discrimination / harassments socially or at the work place?	☐	☐
75) As a women, do you feel the pressure of domination by your family, society etc.?	☐	☐

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