

RICE BRAN AND ITS MAIN COMPONENTS: POTENTIAL ROLE IN THE MANAGEMENT OF CORONARY RISK FACTORS

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[Received July 29, 2004; Accepted September 27, 2004]

ABSTRACT: *The prevention of cardiovascular disease events is related to a complex management of conventional and non-conventional risk factors. The first approach to reduce the cardiovascular disease risk is a correct dietary approach. Rice bran and its main components (Fibres, unsaturated fatty acids, triterpene alcohols, phytosterols, tocotrienols, alpha-tocopherol) have demonstrated their property to improve the plasma lipid pattern of rodents, rabbits, non-human primates and humans, reducing total plasma cholesterol and triglyceride concentration and increasing the high density lipoprotein cholesterol level. Other potential properties of rice bran components have been studied both in vitro and in animal models such as modulation of the pituitary secretion, inhibition of the gastric acid secretion, antioxidant action and inhibition of the platelet aggregation. The main aim of this paper is to review the available data on pharmacology and toxicology of rice bran and its main components with a particular attention to those data suggesting a potential efficacy in reducing the cardiovascular disease risk.*

KEY WORDS: : Cardiovascular disease, gamma-oryzanol, Tocotrienols, Prevention, Rice bran.

ABBREVIATIONS: HMGCoA-R= 3-Hydroxy-3-Methyl-Glutaryl-CoenzymeA reductase; HDL-C= High Density Lipoprotein Cholesterol; LDL-C= Low Density Lipoprotein Cholesterol; PL = Phospholipids; TC= Total plasma Cholesterol; TG= Plasma Triglycerides; VLDL-C= Very Low Density Lipoprotein Cholesterol; GH= Growth Hormone; LH= Luteinizing Hormone; PRL= Prolactin Releasing hormone TSH= Thyroid Stimulating Hormone

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INTRODUCTION

It is well known that cardiovascular death rate is lower in Mediterranean countries than in Northern Europe and North America. Conclusions from these studies acclaimed the Mediterranean diet that is very rich in complex carbohydrates, mono- and polyunsaturated fatty acids, fibres and antioxidants (fruits and fresh vegetables), poor in cholesterol, saturated and oxidised fatty acids, in association with moderate alcohol consumption. The Far Eastern Asian dietary pattern is also associated to a lower mortality rate because of cardiovascular events: it is poor in cholesterol and in fatty acid too, and very rich in rice and soy-derived proteins. Unfortunately, both Mediterranean and Far Eastern Countries are changing their alimentary habit toward the Anglo-Saxon one much more than Anglo-Saxon Countries are getting nearer to Mediterranean or Asiatic diets.

However, studying both diets we can find some nutrients that can be isolated and used as dietary supplements, useful to supply dietary unbalances in order to reduce the cardiovascular disease risk. For instance, there is overwhelming evidence that support the antihypercholesterolemic effect of vegetable oils that are rich in polyunsaturated fatty acids, mainly -linolenic and linoleic acid.

Observational studies on distinct populations support the existence of a linear relationship between plasma lipid levels and cardiovascular disease induced death rate. This is sound to be true even if elevated plasma cholesterol is just a borderline one (Durrington et al. 1999). While pharmacological research has focused on energy in searching for new drugs able to drastically decrease Low Density Lipoprotein Cholesterol (LDL-C) and total triglycerides (TG) plasma level, Italian (Ricci et al. 1997), European (Task Force 1998) and United States of America (Adult Treatment Panel III 2001) guidelines underline the dietary and behavioural habit correction as a first step for the hyperlipoproteinemic patient at risk for cardiovascular events. It is only after this intervention fails to sufficiently lower plasma lipid level, that the beginning of a pharmacological treatment is admitted. Once this starts, it

should become a chronic and continuing therapy. Therefore, these Guidelines do not allow a pharmacological treatment for youth hyperlipoproteinemic subjects (except for Familial Hypercholesterolemia) and moderate dyslipidemias. Very often, these dyslipidemias hide such a Familial Combined Hyperlipoproteinemia, linked to high incidence of cardio- and cerebrovascular disease related death. These diseases have no right to be covered by drug payment exemption, because the plasma lipid levels of these patients are often not high enough (Note CUF 1998). Consequently, chronic prevention therapy appears onerously expensive for patients and even the cost/benefit relationship is still too low to justify starting chronic pharmacological therapy. This is particularly true in cases where there are no other risk factors (e.g. young, sporting, non-smoking subjects, moderately hypercholesterolemic individuals). Beside, thus it is very important to consider the psychological impact that a therapeutic intervention that could have on such a patient. We point out the necessity to find dietary complements in order to help the moderately dyslipidemic patient, particularly the young one, and let him/her obtain proper cardiac disease prevention from a parapharmacological point of view.

Thus, the last Adult Treatment Panel of the National Cholesterol Educational Program suggests introducing in the everyday diet 4 portions of soy proteins (for a total daily amount of 25 g), 2 grams of phytosterols and vegetable soluble fibres (like psyllium, guar, pectin, oat) in order to reduce or maintain an adequate cholesterolemia (Adult Treatment Panel 2001).

This is the context in which rice bran and derived products appears as nutraceuticals potentially useful in the prevention of cardiovascular diseases (Cicero et al. 2001).

RICE BRAN OIL: ORGANOLEPTIC PROPERTIES AND ACTIVE PRINCIPLES

From a marketing point of view, the most available (and adequately investigated) rice bran derived product is the oil made from the pericarp and germ of the *Oryza sativa* seeds. Rice bran constitutes about 10% of rough rice grain and contains 18% to 22% oil. It is pale yellow, limpid (at 20°C), odourless with acid index <0.50, density at 20° between 0.920 and 0.930, refractive index at 20°C between 1.471 and 1.475, smoke point >200°C, pleasant flavour lightly sweet. It contains mainly oleic acid (38.4%), linoleic acid (34.4%) and α -linolenic acid (2.2%) as unsaturated fatty acids, and palmitic (21.5%) and stearic (2.9%) acids as saturated fatty acids (Sayre et al. 1990). In contrast to most common refined vegetable oils, crude rice bran oil contains a rich unsaponifiable fraction (up to 5%) mainly composed by sterols (43%), triterpene alcohols (28%) 4-methyl-sterols (10%) and less polar components (19%) (Sayre et al. 1990). Phytosterols include β -sitosterol (900 mg%), campesterol (500 mg%), stigmasterol (250 mg%), squalene (320 mg%) and γ gamma-oryzanol (1.6%). The so-called gamma-oryzanol, often identified as the active molecule of rice bran oil, is a mixture of ferulic acid esters of triterpene alcohols such as

cycloartenol (106 mg%) and 24-methylene cycloartanyl (494 mg%) (Metwally et al. 1974, Norton 1995), firstly isolated by Kaneko and Tsuchiya in the early 1950s (Kaneko et al. 1955). Its fundamental molecular structure is the ferulic acid aromatic phenolic nucleus esterified to cyclopentanperihydrophenanthrene (Seetharamaiah et al. 1986). Rice bran oil contains a little variable quantity of tocotrienols (from 72 to 612 ppm, especially β and γ -tocotrienols), but it is naturally very rich in tocopherol (ca. 100 mg%), similarly to another vegetable oil with well-known antihyperlipidemic action, the soybean oil (Rukmini et al. 1991, Rogers et al. 1993).

ANTI-HYPERLIPIDEMIC PROPERTIES OF RICE BRAN COMPONENTS

In the last 35 years, numerous studies have been carried out on the effect of rice bran oil and gamma-oryzanol activity on lipid metabolism and oxidation in rats (Fujiwara et al. 1983), rabbits (Fujiwara et al. 1980), hamsters (Kahlon et al. 1992), monkeys (Nicolosi et al. 1990) and humans (Suzuki et al. 1970a).

Studies on rats: Studies carried out on the rat have given interesting but often contrasting results. Shinomiya et al.'s study on gamma-oryzanol partially went against the supposed antiatherogenic property of this compound. The lipid content of the plasma and liver and enzyme activities in the aorta were determined at the 28th, 56th and 84th day of the observation period in rats given a standard diet (I), a high cholesterol diet (II), a high cholesterol diet plus 0.5% gamma-oryzanol (III) or a high cholesterol diet plus 2% gamma-oryzanol (IV). Plasma TC, LDL-C and very low-density lipoprotein-cholesterol (VLDL-C) were significantly higher in rats in groups III and IV than in those in group II. HDL-C was increased in groups III and IV. There were no differences in rat plasma TG or PL concentrations in the four groups. The liver cholesterol ester and triglyceride contents were lower in group IV than in groups II and III. Changes in acid cholesterol esterase activity and acyl-Coenzyme A: cholesterol acyl-transferase activity in rat aorta homogenates to compensate for lipid deposition was greater in groups III and IV than in group II (Shinomiya et al. 1983). In a subsequent study the intravenous administration of gamma-oryzanol and cycloartenol ferulic acid ester 10 mg/kg for 6 days significantly inhibited the increases in serum TC, PL and free cholesterol induced by a high cholesterol diet in male Sprague-Dawley rats. After 12 days of treatment, both substances were able to significantly decrease TG, non-esterified fatty acid, lactate dehydrogenase and transaminases in rapport to the control animals. These results suggest that the intravenous administered gamma-oryzanol and cycloartenol ferulic acid ester could accelerate the excretion of lipids from the blood. However, the same substances administered by oral route for 12 days (100 mg/kg) did not produce significant changes in the effects studied (Sakamoto et al. 1987). Besides these preliminary studies, some well-conducted Indian researches show that rice bran oil and gamma-oryzanol significantly improve the plasma lipoprotein pattern in rat. In fact, Sharma and Rukmini demonstrated that rats

fed with rice bran oil at 10% level for a period of eight weeks showed significantly lower TC, LDL-C and VLDL-C plasma levels, both on cholesterol-containing and cholesterol-free diets. HDL-C was increased, while TG showed a non-statistically significant decrease. Liver cholesterol and triglycerides were also reduced, while faecal excretion of neutral sterols and bile acids was increased (Sharma et al. 1986, Sharma et al. 1987). Moreover, the serum total, free esterified and non-HDL-C levels of rats maintained on a 10% refined rice bran oil diet were significantly lower than those on a 10% groundnut oil diet; HDL-C showed a tendency to be higher. Liver lipids of rats fed rice bran oil were also markedly lower than in their groundnut oil fed counterparts. Addition of gamma-oryzanol at 0.5% level to the diet containing rice bran oil showed a further significant decrease in serum TC, but not of liver lipids (Seetharamaiah et al. 1988, Seetharamaiah et al. 1989). Feeding phytosterols, cycloartenol and 24-methylene-cycloartenol in amounts present in rice bran oil unsaponifiable matter to hypercholesterolemic rats for 8 weeks indicated that cycloartenol alone significantly reduced both plasma cholesterol and TG levels (Rukmini et al. 1991). Besides, the effects of feeding two levels of rice bran oil (5% and 20% of the diet) on growth, plasma and liver lipid parameters of Wistar rats, were compared with those produced on animals which had been fed by the same quantity of peanut oil. There was no significant difference with respect to the organ weights between control and experimental groups. Animals, which received rice bran oil in their diet, had, in general, comparatively lower levels of TC, TG and PL. On the other hand, animals receiving 20% rice bran oil in their diet, showed an increase of 20% in HDL-C, within 18 weeks ($p < 0.05$), when compared to the animals fed with peanut oil. Similarly, LDL-C and VLDL-C were lower in rice bran oil fed groups, than in the peanut oil fed groups. There was, however, no significant difference in the plasma cholesterol/phospholipid ratio or in the polyunsaturated/saturated fatty acids ratio, nor any in the oleic/linoleic, oleic/stearic, palmitoleic/palmitic, oleic/palmitic, and oleic/palmitoleic ratios between the two groups (Purushothama et al. 1995).

Having assessed the antihyperlipidemic property of rice bran oil, it has been studied the possibility of increasing its cost/efficacy ratio by mixing it with other less expensive vegetable oils rich in polyunsaturated fatty acid has been examined. Rats fed with rice bran oil plus safflower oil and sunflower oil in 70:30 ratio for a period of 28 days showed significantly ($p < 0.05$) lower levels of TC, TG and LDL-C and increased HDL-C in animals fed by a high cholesterol diet and cholesterol free diet. Liver cholesterol and TG were also reduced, while faecal excretion of neutral sterols and bile acids was increased with the use of rice bran oil blends. Moreover, the high rice bran oil content of tocopherols and tocotrienols may improve the oxidative stability of the blends. Thus, in addition to improving the plasma lipid profile, blending of rice bran oil with other oils can result in an economic advantage of lower prices (Sunitha et al. 1997). A somewhat contrasting effect was recently observed by Koba et al. who studied the

influence of a 0.5% cholesterol diet supplementation on rice bran oil's antihyperlipidemic effect. This research examined the effect of cholesterol supplementation on the cholesterol-lowering ability of different rice bran oil/safflower oil blends. Male Sprague Dawley rats (4 wk old) were fed with purified diets containing 10% fat with or without the addition of 0.5% cholesterol for 3 wk. The fat was either safflower oil or rice bran oil alone or a mixture of these two oils at ratios of 7:3, 5:5 or 3:7. Without cholesterol supplementation, there were no significant differences in the serum and liver cholesterol levels among rats fed different dietary fats. However, the HDL-C level of rats fed the rice bran oil-containing diets (especially in rats fed by the 3:7 diet) was higher than that of rats fed with the diet containing safflower oil alone. This resulted in an increase in the ratio of HDL-C/TC, a desirable outcome. Supplementation of the diets with 0.5% cholesterol significantly increased the cholesterol level in both serum and liver. Increasing the proportion of rice bran oil in the diet further raised the plasma TC level, whereas it reduced liver cholesterol. In the case, the specific effect of the 3S/7R mixture on the ratio of HDL-C/TC disappeared. An explanation for this phenomenon is that smaller percentages of polyunsaturated fatty acids in the rice bran oil containing diets than in the safflower oil diet might have reduced their ability to dispose the circulating serum cholesterol into the liver (Koba et al. 2000). Finally, a Chinese Author reported that chronic oral administration of gamma-oryzanol is able to reduce the development of experimental coronary atherosclerosis in rats fed a hypercholesterolemic diet from the birth (Zhang 1986), however the design of this study carried out on few animals is not clearly understandable. However, rice oil and gamma-oryzanol supplementation seems to be strongly related to an improvement of the cardiovascular disease risk profile of rats, especially when they are submitted to a fat diet (Edwards et al. 1994, Radcliffe et al. 1997). If the reduction of the liver lipid content could be demonstrated even in humans, it might be a very useful mean to treat the non alcoholic steatohepatitis, that appears to be strongly associated both to metabolic syndrome and cardiovascular disease (Marchesini et al. 2003).

Studies on rabbits and hamsters: gamma-oryzanol's effect on the development of atherosclerosis was tested in 18 male New Zealand white rabbits fed a diet containing 1% cholesterol with or without 1% gamma-oryzanol for 10 weeks. The antiantheromatic effect was assessed by the degree of resistance of LDL-C to oxidation by copper sulphate and the effect of copper oxidised LDL-C on the incorporation of oleate into cholesteryl ester by peritoneal macrophages. In addition, the plasma TC, TG and lipid peroxide levels were measured before, during and at the end of cholesterol loading. It was found that oleate incorporation into cholesteryl ester by macrophages was significantly reduced in the gamma-oryzanol-treated group compared to the non-treated group, and that the reduction was via a mechanism independent of anti-oxidant action. However, no differences were found in plasma TC, LDL-C, TG and lipid peroxides levels or in the areas of atherosclerotic plaques between the two groups.

Thus, it was concluded that gamma-oryzanol has little or no preventive effect on atherosclerosis in rabbits (Hiramatsu et al.1990). However, in an other study conducted in order to determine the relative cholesterol-lowering effects of several levels of full-fat rice bran in 4 week old hamsters, the separate effects of defatted rice bran and/or crude rice bran oil were investigated at levels that were equivalent to those present in 43.7% full-fat rice bran. After 21 days, in cholesterol-fed hamsters total liver cholesterol content was significantly lower in those fed the defatted rice bran diet with rice bran oil compared with those fed the cellulose diet. Full-fat rice bran was the only treatment that significantly lowered both plasma and liver cholesterol (Kahlon 1992). Moreover, the antiatheromatic action of gamma-oryzanol was investigated in 32 hamsters made hypercholesterolemic by feeding chow-based diets containing 5% coconut oil and 0.1% cholesterol with or without 1% gamma-oryzanol for 7 weeks. Related to the control animals, gamma-oryzanol treatment resulted in a significant reduction in plasma TC (28%, $P < 0.01$) and in the sum of Intermediate Density Lipoprotein Cholesterol, LDL-C and VLDL-C (non-HDL-C) (34%, $P < 0.01$). In addition, the gamma-oryzanol-treated animals also exhibited a 25% reduction in percent cholesterol absorption versus control animals. Endogenous cholesterol synthesis, as measured by the liver and intestinal HMGCoA-R activities, showed no difference between the two groups. To determine whether a lower dose of gamma-oryzanol was also efficacious and to measure aortic fatty streaks, 19 hamsters were divided into two groups and fed for 10 week chow-based diets containing 0.05% cholesterol with 10% coconut oil (control) and the control diet plus 0.5% gamma-oryzanol. Relative to the control, gamma-oryzanol-treated hamsters had reduced plasma TC (44%, $p < 0.001$), Non-HDL-C (57%, $p < 0.01$), and TG (46%, $p < 0.05$) concentrations. Despite a 12% decrease in HDL-C ($p < 0.01$), the gamma-oryzanol-treated animals maintained a more optimum non-HDL-C/HDL-C profile (1.1 ± 0.4) than the control (2.5 ± 1.4 ; $p < 0.01$). Aortic fatty streak formation, so defined by the degree of accumulation of oil red O-stained macrophage-derived foam cells, was reduced by 67% ($p < 0.01$) in the gamma-oryzanol-treated animals (Rong et al. 1992). Furthermore, other studies conducted on animals fed different models of hyperlipidemic diets showed a significant reduction in lipoprotein plasma levels (Srinivasan et al.1989, Seetharamaiah et al.1988). As observed in rats, studies on hamster and rabbits confirm the gamma-oryzanol's property to improve the cardiovascular disease risk profile of animals.

Studies on non human primates: Lipid metabolism in rodents is very different to that in humans (e.g. most of the circulating cholesterol is in the form of HDL-C and much less of LDL-C), therefore it is difficult to extrapolate findings in rodents to humans. However, the hypolipidemic response of rice bran oil was investigated in non-human primates fed semi-purified diets containing blends of oils which included rice bran oil at 20-25% Kcal as dietary fat. The study (Nicolosi et al.1991) demonstrated that the degree of

serum TC and LDL-C reduction was highly correlated with initial serum cholesterol levels in monkeys fed a standard diet. Further, rice bran oil supplementation in the diet significantly influenced serum TC, LDL-C and apolipoprotein B causing up to a 40% reduction in LDL-C without significantly affecting apolipoprotein AII and HDL-C plasma levels when rice bran oil was the sole dietary oil. Data showed that cholesterol-lowering capabilities of rice bran oil were not explained by its fatty acid composition.

Studies in humans: The first scientific communication about rice bran oil's antihyperlipidemic property in humans was published by Suzuki and Oshima in 1970 (Suzuki et al. 1970b). They observed in healthy young women that the daily consumption of 60 g of a combination of 70% rice bran oil and 30% safflower oil could more effectively lower plasma TC levels even within 7 days than the respective oils alone, or their combination in other proportions. Moreover, Tsuji and her colleagues observed that the blended oil exerted a hypocholesterolemic effect on 7 young female volunteers even when five eggs were daily consumed for 7 consecutive days, associated with a significant increase of plasma HDL-C level (Tsuji et al.1989). It was only in 1982 that Ishihara et al. reconsidered their above mentioned results, when they tested gamma-oryzanol on 40 women affected by post-menopausal syndrome (Ishihara et al.1982). After 4-8 treatments of 300 mg of gamma-oryzanol/day there were decreases in TC, LDL-C and TG plasma levels as statistically significant as an increase of HDL-C concentration in hyperlipoproteinemic subjects. Besides, plasma lipid peroxide level was significantly reduced in those subjects who had previously elevated levels. During this study, no side effects have been recognised and no particular change in liver and renal function tests was found. The same results were subsequently confirmed in a wider female population (Ishihara 1984). In another study twelve moderately non-obese hyperlipoproteinemic subjects (mean baseline TC and TG = 247.3 ± 10.55 mg/dl and 349.8 ± 42.41 mg/dl, respectively) were advised to substitute cooking oils that they normally employed with rice bran oil. Their plasma lipid levels were compared with those of 11 patients with a similar baseline lipid pattern. The rice bran oil treated patients showed a 16% and 25% decrease in plasma TC, after 15 and 30 days of treatment, respectively, in respect to the control group. The rice bran oil treated group also showed a 32% and a 35% reduction of plasma TG, after 15 and 30 days of administration, respectively. The decrease in lipid levels was faster in subjects with higher baseline levels (Raghurma et al. 1989). Similar results were found in other studies where gamma-oryzanol was compared to probucol. When 300 mg/day gamma-oryzanol was administered for three month to hyperlipidemic subjects a significant decrease in plasma TC and LDL-C was observed in both hypercholesterolemic and hypertriglyceridemic patients, while a relevant increase in HDL-C was caused only in the hypercholesterolemic group. In all the above mentioned studies no side effect were observed (Yoshino et al.1989a, Yoshino et al.1989b). Moreover, 20 chronic schizophrenic patients with dyslipid-

emia (TC \geq 220 mg/dl, TG \geq 150 mg/dl, or HDL-C \leq 40 mg/dl) who had been receiving neuroleptics for a mean of ten years were treated with 100 mg of gamma-oryzanol three times daily for 16 weeks. Mean TC and LDL-C levels, respectively, decreased significantly, from 204 mg/dl and 124 mg/dl at baseline to 176 mg/dl and 101 mg/dl at week 12. Mean HDL-C levels were 36.1 mg/dl at baseline and 35.9 mg/dl at week 12. Mean apolipoprotein B levels decreased significantly from 116 mg/dl to 101 mg/dl at week 16, while apolipoprotein A-II levels increased from 31.7 mg/dl to 34.7 mg/dl and apolipoprotein B/apolipoprotein A-I ratio declined from 0.99 to 0.84. Once again, no treatment side effects were recorded (Sasaki et al.1990). Furthermore, the effect of rice bran oil and oils not commonly consumed in the United States, on plasma lipid and apolipoprotein concentrations was studied within the context of a NCEP Step 2 diet and compared with the effects of canola, corn and olive oils. The study subjects were 15 middle-aged and elderly subjects (8 postmenopausal women and 7 men; age range, 44 to 78 years) with elevated LDL-C (range, 133 to 219 mg/dL). Diets enriched in each of the test oils were consumed by each subject for 32-day periods in a double-blind fashion and were allocated in a Latin square design. All food and drink were provided by the metabolic research unit. Diet components were identical (17% of calories as protein, 53% as carbohydrate, 30% as fat [$<7\%$ as saturated fat], and 80 mg cholesterol/1000 kcal) except that two thirds of the fat in each diet was contributed by rice bran, canola, corn or olive oil. Mean \pm SD plasma TC concentrations were 192 \pm 19, 194 \pm 20, 194 \pm 19, and 205 \pm 19 mg/dL, and LDL-C concentrations were 109 \pm 30, 109 \pm 26, 108 \pm 31, and 112 \pm 29 mg/dL after consumption of the rice bran, canola, corn and olive oil-enriched diets, respectively. Thus, plasma TC and LDL-C concentrations were similar and statistically indistinguishable when the subjects consumed the rice bran, canola and corn oil-enriched diets (Lichtenstein et al.1994). A double blind 12-week study was performed to investigate the effect of a tocotrienol rich fraction obtained by molecular distillation from specially processed rice bran oil on cardiovascular disease risk factors on hypercholesterolemic human, subjects (TC $>$ 220 mg/dl). After acclimatisation to an alcohol free regimen, participants were assigned to the NCEP Step-1 diet (saturated fat $<19\%$, total fat $<30\%$ of total calories and cholesterol <300 mg/day). After four weeks, 21 subjects was continued on the standard NCEP Step-1 diet, while a group of 20 added to this diet 200mg/day of a tocotrienol mix (12.5% α -tocotrienol, 21% γ -tocotrienol, 10% δ -tocotrienol, 4.5% β -tocotrienol, 17% P_{2,5}-tocotrienol, 18% unidentified tocotrienols). After additional four weeks, the treated group showed a significant decrease ($p<0.05$) in plasma TC and LDL-C levels (-12% and -16%, respectively, versus -2% and -3% of the control group). Moreover significant decreases ($p<0.05$) in serum apolipoprotein B, lipoprotein (a) (Lp(a)), platelet factor 4 and thromboxane B₂ were observed in connection with the baseline level (respectively, -15%, -17%, -14% and -31%) (Qureshi et al.1997). It is remarkable that the tocotrienol treatment in this studies caused a decrease of

Lp(a) plasma level when neither diet nor antihypercholesterolemic drugs are likely to have an impact on it. A combined treatment with NCEP Step-1 diet and tocotrienol rich fraction of rice bran has also determined a 25% reduction in LDL-C plasma level of hypercholesterolemic subjects in a recent study carried out by Qureshi et al. (2002). Finally, we would like highlight a recent case involving a 9 year old Caucasian patient. She is affected by IIb Fredrickson type of dyslipidemia (TC $>$ 200mg/dl, TG $>$ 200mg/dl) that is genetic based. After one-month of treatment with rice oil (20 g/day) and polyunsaturated fatty acids (650 mg/day), the child showed a significant decrease of TC, LDL-C, TG and Lp(a) reaching her age standard values (Cicero et al.2001). We resumed the main findings of studies carried out on the effects of rice bran oil and its principal components on the lipid metabolism in Table 1 and 2. It seems that rice bran oil and its main components are able to safely improve the plasma lipid pattern of hypercholesterolemic patients, however the quality of the published studies is often poor and the number of tested subjects really slight. Thus it would be necessary to carry out new randomised clinical trials on more wide population to conclude definitively if rice oil could be considered a safe and long-term efficacious treatment for mild-moderate hyperlipoproteinemias.

MECHANISM OF ACTION: SOME HYPOTHESES

The mechanism of action of rice bran and the derived oil on lipid metabolism is not yet completely evident. Its specific content of polyphenols (gamma-oryzanol), phytosterols, tocopherols and tocotrienols is supposed to contribute to antihyperlipidemic action, while the particular fatty acid mono- and polyunsaturated composition seems not to be fundamental in its activity (Rong et al.1992). Moreover, the additional improvement on lipid metabolism improvement by rice bran oil/safflower oil blends (Kobe 1997) can not be easily explained by their fatty acids or plant sterols composition, because the blending of rice bran oil with sunflower oil (a vegetable oil with a biochemical composition really similar to the safflower oil) did not exerted the same antihypercholesterolemic property (Sugano et al. 1997). The fact that rice oil isolable substances are altogether responsible for global effect on plasma lipid, is the most probable hypothesis.

Gamma-oryzanol: Possible fundamental antiatherosclerotic role rest on gamma-oryzanol (Kanbara et al. 1992) (Figure 1). In different animal models it was found that rice bran oil and its unsaponifiable matter significantly increase the faecal excretion of acid and neutral sterols (Seetharamaiah et al. 1989). For example, gamma-oryzanol's effect on biliary secretion and faecal excretion of cholesterol, PL and bile acids was examined in male albino rats. Feeding gamma-oryzanol at 0.5% level with the control diet did not cause any change in bile flow and composition. On feeding gamma-oryzanol with high cholesterol diet, the bile flow and total bile acid output were increased by 12% and 18% respectively, as observed by the significant increase in the faecal excretion of cholesterol (28%) and bile acids (29%), whereas cholesterol absorption

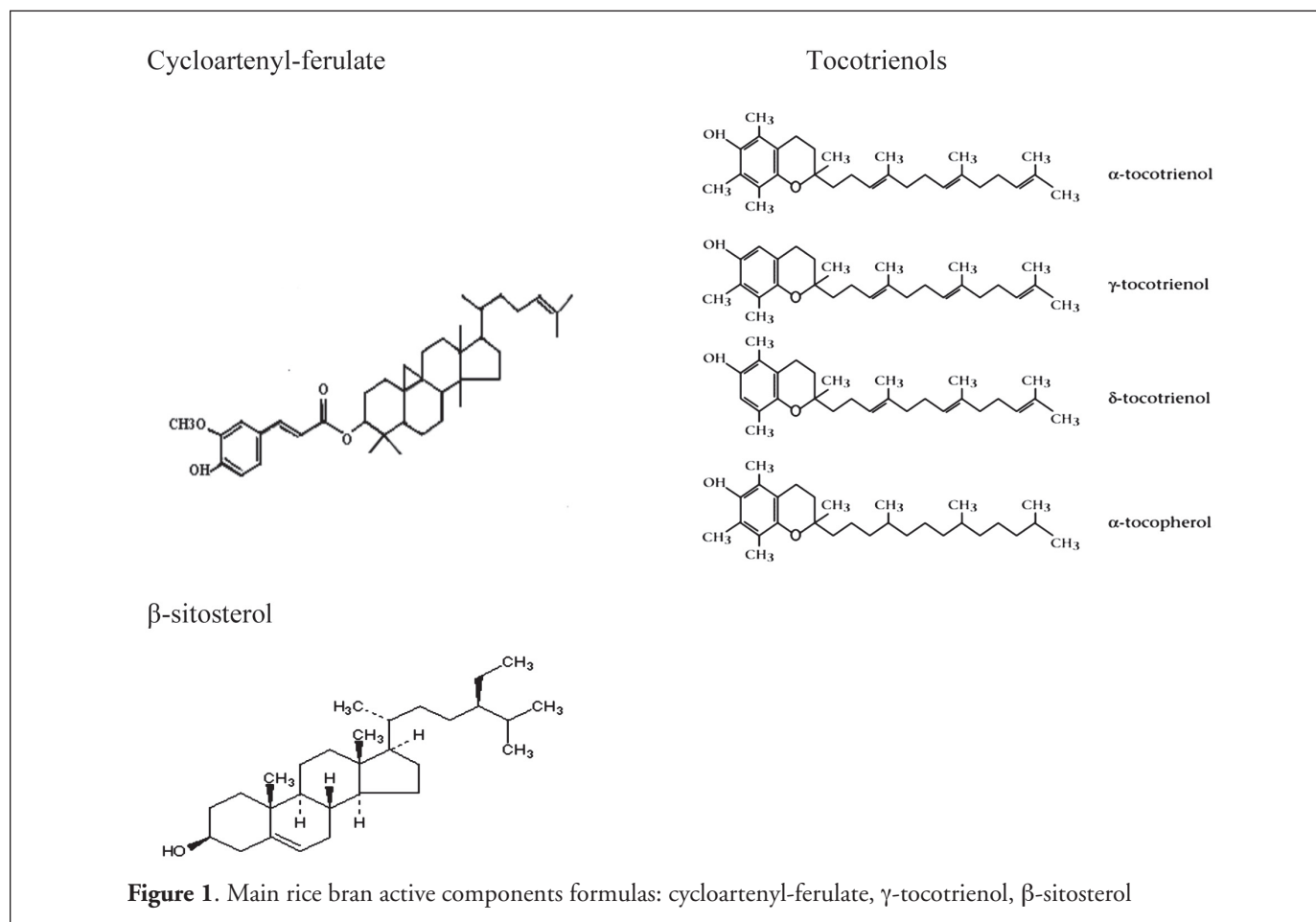
Table 1 – Rice bran derived products effects on main lipid metabolism parameters of different animal species

Animal species	Rice bran derived products	Dose (mg/kg, or % of dietary fats)	Administration route	Pharmacological effect	Ref.
Rats	γ -oryzanol	0,5-2%	OS	↓ TC, LDL-C, HDL-C, VLDL-C, liver cholesterol esters & triglycerides	Shinomiya, 1993
Rats	γ -oryzanol	10 mg/kg	IV	↓ TC, PL, TG, Non-esterified fatty acids & Free cholesterol	Sakamoto, 1997
	γ -oryzanol	1%	OS	↓ TC, LDL-C, VLDL-C, TG, PL, ↑ HDL-C	Seetharamaiah, 1988
Rats	Cycloartenol ferulate	10 mg/kg	IV	↓ TC, PL, TG, Non-esterified fatty acids & Free cholesterol	Sakamoto, 1997
Rats	Rice Bran Oil	10%	OS	↓ TC, LDL-C, VLDL-C, TG, PL, ↑ HDL-C, ↓ Liver cholesterol & triglycerides, ↑ Faecal bile acids & neutral sterols	Sharma, 1987; Seetharamaiah, 1989
Rabbits	γ -oryzanol	1%	OS	↓ oleate incorporation into cholesteryl ester by macrophages	Hiramatsu, 1990
Hamsters	γ -oryzanol	1%	OS	↓ TC, LDL-C, VLDL-C, TG, HDL-C, cholesterol absorption, Aortic fatty streak formation	Rong, 1997
Monkeys	Rice Bran Oil	20-25%	OS	↓ TC, LDL-C, Apolipoprotein B	Nicolosi, 1991

Table 2 – Rice bran derived products effects on main lipid metabolism parameters observed in clinical trials

Rice bran derived products	Daily dose	Study Design (Study duration)	Number of subjects	Pharmacological effect	Ref.
γ -oryzanol	300 mg	RCT* (8 weeks)	40 Post-menopausal women	↓ TC, LDL-C, TG, Lipid peroxides, ↑ HDL-C	Ishihara 1982
	300 mg	RCT (8 weeks)	80 Post-menopausal women	↓ TC, LDL-C, TG, Lipid peroxides, ↑ HDL-C	Ishihara 1984
	300 mg	RCT (13 weeks)	80 Hypercholesterolemics	↓ TC, LDL-C, TG	Yoshino, 1989
	300 mg	RCT (8 weeks)	20 Hypercholesterolemics	↓ TC, LDL-C, Apolipoprotein B,	Sasaki, 1990
	500 mg	RCT (9 weeks)	40 Healthy young men	No effect on metabolic parameters	Fry, 1997
Rice-derived Tocotrienols	200 mg	OS (12 weeks)	41 Hypercholesterolemics	↓ TC, LDL-C, Apolipoprotein B, Lp _a , Platelet's aggregation	Qureshi 1997
Rice Bran Oil	42 g	Cross-Over (14 weeks)	10 young healthy females	↓ TC, LDL-C	Suzuki, 1970
	50 g	RCT (4 weeks)	23 Mixed hyperlipoproteinemics	↓ TC, LDL-C, TG	Raghuram, 1989
	50 g	Cross-Over (14 weeks)	10 Young healthy females	↑ HDL-C	Tsuji, 1989
	50 g	Cross-Over (5 weeks)	15 Hypercholesterolemics	↓ TC, LDL-C	Lichtenstein, 1994

*RCT = Randomized Clinical Trial



was lowered by 20% (Seetharamaiah et al. 1990). It is possible that gamma-oryzanol's antihypercholesterolemic effect is partially due to its sterol moiety, which is partly split off from the ferulic acid part in the small intestine by cholesterol esterase (Swell et al. 1954, Sugano et al. 1997). Anyway, in 1987 Sakamoto et al. underlined the fact that gamma-oryzanol and cycloartenol ferulate have an antihyperlipidemic action and this is more remarkable by intravenous than by oral administration (Sakamoto et al. 1987), maybe due to a direct inhibition of lipid metabolism. Moreover, ferulic acid alone, that is adsorbed and metabolised, has shown an intrinsic hypolipidemic effect in some studies (Sharma 1980, Seetharamaiah et al. 1993). The pharmacological mechanism of the observed effect is still unknown, because no direct inhibition on HMGCoA-R was observed (Seetharamaiah et al. 1989). Rice bran oil's antiatherogenic action could also be based on other mechanisms, for example cholesterol-esterase inhibition by cycloartenol, or by the inhibition of the accumulation within macrophages of cholesterol-esters or by the modulation of cholesterol acid esterase and acyl-CoA-cholesterol-acyltransferase by gamma-oryzanol (Rukmini et al. 1991).

Phytosterols:

Three groups of phytosterols are present in crude rice bran oil: 4,4'-dimethylsterols (1.2%), 4-monomethyl-sterols

(0.4%) and 4-desmethylsterols (1.8%) (Figure 1) (Sayre et al. 1990). It is well known that for phytosterols, the active principles in the antihyperlipidemic action of Soya-derived products, less than 5% is absorbed through intestinal mucosa on oral intake (Wilson et al. 1998), with the majority being excreted in the stool. This is the reason why it has been proposed that rice bran oil's action could be based on bile acids and TC complexation in the intestinal lumen (anion exchange resin-like action). This hypothesis seems to be confirmed by studies that showed a high faecal excretion of TC and bile acids in rats that underwent a hyperlipidemic diet (Shinomiya et al. 1983), although a very recent research has contradicted the same result (Koba et al. 2000). However, the phytosterol antihypercholesterolemic effect has been clearly observed in different studies on human subjects, even when tested at low dosage (Wang et al. 1999). Moreover, cycloartenol and 24-methylcycloartanol magnify the cholesterol-lowering potential of soy sterols at a very low dietary level, presumably increasing the faecal extraction of neutral and acidic sterols (Kiribuci et al. 1983). A similar effect, even if less evident, was observed with a combination of cycloartenol and β -sitosterol in rats (Ikeda et al. 1985).

Tocotrienols: More recently, the attention of researchers has mainly focused on tocotrienols as probable main mediators of

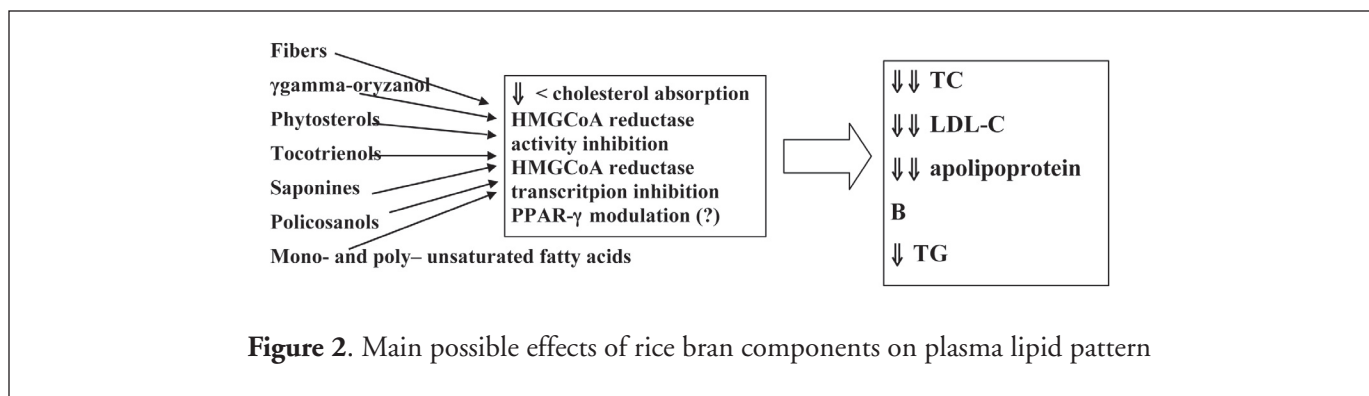


Figure 2. Main possible effects of rice bran components on plasma lipid pattern

the rice bran oil antihypercholesterolemic effect. Tocotrienols are naturally occurring farnesylated unsaturated analogues of α -, β -, γ - and δ -tocopherol (Vitamin E) (Figure 1). In contrast to corn, wheat, and soybean (contain mainly tocopherols), barley, oats, palm, and commercial rice brans and rice bran oil contain until 70% tocotrienols, which consists of α -, β -, γ - and δ -tocotrienols (Raghuram et al.1995). Their hypocholesterolemic activity has been clearly demonstrated in different animal species (Pearce et al.1992, Hood et al.1992) and in humans (Lichtenstein et al.1994, Qureshi et al. 1991). Intravenously administered, they are able to inhibit the HMGCoA-R, a key enzyme in the endogenous synthesis of cholesterol, through two post-transcriptional actions, increasing the controlled degradation of reductase protein and decreasing the efficiency of the translation of HMGCoA-R messenger RNA (Khor 1995, Parker 1993). On the other hand, α -tocopherol appears to induce HMGCoA-R activity (Qureshi et al.1996). Tocotrienols have also shown an antithrombotic, antiproliferative and antioxidant activities (Watkins et al.1993, Komiyama et al.1992). What is really interesting is that tocotrienols appear to inhibit atherosclerotic lesions development in atherosclerosis prone mice because of apolipoprotein E deficiency, at least partly independently from plasma lipid reduction (Qureshi et al.2001). However, not all clinical trials confirm the antihypercholesterolemic activity of tocotrienols in humans and some Authors suggest prudence in this claim (Kerckhoffs et al. 2002; Mustad et al. 2002).

In conclusion, at present it is not possible to fix an unequivocal mode of action for rice bran oil because of the heterogeneity of the available data (Figure 2). Nevertheless, there is no doubt about the existence of an antihyperlipidemic action by rice bran oil components (Sugano et al.1993).

NON-ANTHYPERLIDEMIC EFFECTS OF MAIN RICE BRAN OIL COMPONENTS.

Obviously, the acceptability of a nutraceutical potentially useful for the prevention of cardiovascular disease is even more relevant when possible effects on other apparatus are also known. First studies about rice bran oil and its active components' (especially γ -oryzanol) pharmacological properties took place in the 1960's (Nakamura 1966). Therefore, after the initial and rather ignored observation of Suzuki et

al. about rice bran oil's effects on human lipid pattern versus other fatty acids and comestible oils (Suzuki et al.1970b), different researchers looked at potential neuroendocrinological, gastroenterological anabolic and dermatological effects of γ -oryzanol.

Neuroendocrinological studies: Different studies on a modulating action on anterior pituitary hormone secretion (Ishihama et al.1966, Yamauchi et al.1981, Takayanagi et al.1981) have been run. The main results of these researches maintain that γ -oryzanol can be a LH, TSH, GH and PRL secretion inhibitor. The inhibition of the LH secretion after a single intravenous injection was significantly stronger than PRL's one in normal male rats and ovariectomized female rats (Yamauchi et al.1980). A decrease in TSH production was observed in human patients affected by hypothyroidism both acutely and chronically treated with γ -oryzanol 300mg/day (Shihomura et al.1980). A single subcutaneous (s.c.) injection of γ -oryzanol 20 mg/kg suppressed GH synthesis and PRL release one hour after the injection. Moreover, it stimulated the tyrosine hydroxylase activity resulting in an increase in the dopamine content of the medial basal hypothalamic nucleus (MBN), then reduced by a treatment with α -methyl-p-tyrosine, a selective tyrosine hydroxylase inhibitor. γ -oryzanol did not alter norepinephrine synthesis, while it significantly increased the norepinephrine release in MBN. These results may explain the data concerning the changes in GH and PRL serum levels by γ -oryzanol and also suggest that it can affect the synthesis and/or release of at least two hypothalamic neurotransmitters, dopamine and norepinephrine, resulting in alterations of anterior pituitary hormone synthesis and/or release (Ieiri et al.1982). However, norepinephrine content in different rat brains slightly but significantly increased when 100 mg/kg γ -oryzanol was given s.c. once daily for 1, 5 or 10 days. The turnover rate of brain norepinephrine tended to decrease with the administration of γ -oryzanol. From the results, it is likely that successive doses of γ -oryzanol increase brain norepinephrine by inhibiting degradation or release of norepinephrine (Kaneta et al.1979). Finally, Hiraga et al. have showed in a wide variety of tests that phytosterol cycloartenol ferulic acid ester, a component of γ -oryzanol, has a suppressant effect on the central nervous system. Rats treated with the higher dose of cycloartenol ferulic acid ester (1000

mg/kg) appeared significantly sedated; the drug was associated with more resistance to pentylenetetrazol-induced convulsion, increase of avoidance latency after electroconvulsive shock-induced amnesia and improvement of brain glucose metabolism under experimental cerebral ischemia (Hiraga et al.1993). On the other side, in different tests carried out on mice and rats (Kim et al.2002), Kim et al. observed that fermented rice bran has a significant anti-stress and anti-fatigue effect. The neuroendocrinological action of gamma-oryzanol have not been further studied on different animal models or in human, thus our knowledge in this field are really limited. However, they can at least partly justify the gamma-oryzanol efficacy in reducing the perimenopausal symptomatology in two randomised clinical studies carried out on Asian women in the late '60s (Ishihara et al.1982, Ishihara 1984). And it is widely accepted that the cardiovascular disease risk of women increases exponentially with the menopause.

Gastroenterological studies: One of the most investigated properties of gamma-oryzanol is its anti-ulcerogenic property (Mizuta et al.1978, Ishihara et al.1984). Different studies have been carried out on rat models to evaluate which pharmacological mechanism is mainly involved in the gamma-oryzanol's anti-ulcerogenic property. The drug, given at 1 to 100 mg/kg s.c. daily for five days, reduced the water-immersion stress ulcer index dose-dependently and slightly prevented the rate of increase in serum levels of 11-hydroxy-corticosterone. These effects were observed in adrenalectomized as well as sham operated rats. Thus, it seemed like that the anti-ulcer action of gamma-oryzanol is due to participation of the autonomic nervous system, but not the hypophysis-adrenal axis (Itaya et al.1976). In male Wistar rats, a 8-day treatment with 100 mg/kg gamma-oryzanol (s.c.), showed a significant inhibition of fasting ulcer, while 5-day pre-treatment exerted slight effects on ulcers induced by pylorus-ligation or stress-atropine. Ten-days treatment with the same dose of gamma-oryzanol in acetic acid induced ulcers lowered serum gastrin levels. Treatment with reserpine prior to stress loading abolished the anti-ulcer effect of 100 mg/kg gamma-oryzanol (s.c.) given for 5 days in stress ulcer. Administration of L-DOPA or 5-Hydroxy-Tryptophan, however, revealed a tendency toward restoration of the anti-ulcer effect of gamma-oryzanol. The conclusion of these observations was that the monoaminergic neuron system is involved in gamma-oryzanol's anti-ulcer action (Itaya et al.1976b). Moreover, gamma-oryzanol was slightly effective as an inhibitor for histamine-stimulated acid secretion, non effective for carbachol-stimulated secretion and significantly inhibited tetragastrin-stimulated secretion. The effect of gamma-oryzanol on acid secretion stimulated by tetragastrin was prevented by vagotomy but not by splanchnicotomy. Thus, it has been assumed that the gastric antisecretory effect of gamma-oryzanol is mediated by the vagus nerve that induces gastrin release (Mizuta et al.1976). This hypothesis is confirmed by previous data that showed a reduction in gastrin release induced by gamma-oryzanol administration in the rat (Itaya et al.1977) and by another study in which the inhibitory effects of gamma-oryzanol and

atropine on gastric secretion were compared using insulin and 2-deoxy-D-glucose as vagal stimulants: s.c. pre-treatment with 100 mg/kg gamma-oryzanol for five days depressed the gastric secretion stimulated both by insulin and 2-deoxy-D-glucose, but the potency was less than that with 10 mg/kg s.c. atropine (Mizuta et al.1978b). gamma-oryzanol's anti-ulcerogenic effect was also studied in mice submitted to conditioned emotional stimuli (communication box method) and Rapid Eye Movement (REM) sleep deprivations (flower pot method). The incidence of gastric lesions in responder mice induced by conditioned emotional stimuli was reduced by twice p.o. administrations at 6 hr intervals of gamma-oryzanol at 200 and 500 mg/kg, oxazolam at 2 mg/kg and atropine at 1-10 mg/kg. The incidence in sender mice was also reduced by gamma-oryzanol at 200 and 500 mg/kg. In addition, the incidence of gastric lesions induced by REM sleep deprivation was also reduced by single administration of gamma-oryzanol at 100 and 200 mg/kg and oxazolam at 5 mg/kg. Moreover, the facilitation of small intestinal propulsive activity in responder mice induced by convulsive electroshock was suppressed by gamma-oryzanol at 100 and 200 mg/kg and atropine at 10 mg/kg (Ichimaru et al.1984). This modulatory effect on gastrointestinal motility was also observed in the dog, too (Mizonishi et al.1980). Furthermore, rice bran oil contains a high percentage of unsaturated fatty acids that act as precursors in the synthesis of arachidonic acid, which is in turn the essential precursor of prostaglandins established inhibitors of gastric secretion. Rice bran oil also contains antioxidants such as α -tocopherol, which may likewise stimulate the synthesis of prostaglandins. Rice bran oil likely acts by increasing prostaglandin output, thus interfering with gastric HCl production (Lloris et al.1991). Finally, a study carried out by Arai in 1982 showed that gamma-oryzanol is able to significantly improve dyspepsia symptoms even in human patients (Arai 1982). In conclusion, gamma-oryzanol and rice bran oil anti-ulcerogenic effect have been adequately studied in different animal model, but not systematically in humans. The employment of a dietary oil to prevent and attenuate gastric hypersecretive disorders could be really useful, thus further studies are needed to establish the real efficacy of rice bran oil in this field.

Studies in other fields: Although gamma-oryzanol is widely employed in the cosmetic industry as an antioxidant, only one scientific study is available in literature about its modulating effect on sebaceous gland secretion after topical application (Ueda et al.1976). Moreover, gamma-oryzanol is widely employed as an anabolic agent by bodybuilding athletes (Rosenbloom et al.1992, Grunewald et al.1993). In fact, gamma-oryzanol is being consumed in the belief that it may elicit anabolic effects ranging from increased testosterone production and release to stimulating human growth hormone release. However, published scientific studies suggest that this molecule is poorly absorbed after oral administration (Cicero et al.2001). Furthermore, animal studies indicate that when these compounds are injected subcutaneously or intravenously, they induce anti-anabolic or catabolic activity.

Table 3 – Main characteristics of a drug useful for cardiovascular disease prevention in population and their applicability to rice bran oil

<u>Characteristics</u>	<u>Rice bran oil</u>
No acute nor chronic toxicity	Tested in both <i>in vitro</i> and animal models
No mutagenicity/carcinogenicity	Tested in both <i>in vitro</i> and animal models
No side effects	Short term safety confirmed in humans, No data available on long-term efficacy
Efficacy	Short term efficacy confirmed in humans, No data available on long-term efficacy
Efficacy on associated diseases	Menopausal symptoms, Dyspepsia
Palatability	Good
Cheapness	Good
Availability	Different in different countries

As reported above, intravenous or subcutaneous injections of gamma-oryzanol in rats have been shown to suppress LH release, reduce GH synthesis and release, and increase release of the catecholamines, dopamine and norepinephrine, in the brain. Although it had still not been directly measured, this metabolic milieu may actually reduce testosterone production (Wheeler et al.1991). In a recent study Fry et al tested the effectiveness of 500 mg/day γ -oryzanol oral supplementation on weight-trained males randomly divided into supplemented and control placebo groups. Test batteries were administered before, after 4 weeks and after 9 weeks of a scheduled resistance exercise program. Both groups demonstrated significant increases in 1 repetition maximum muscular strength (bench press and squat) and vertical jump power, with no differences between the groups. No differences between groups were observed for measures of circulating concentrations of hormones (testosterone, cortisol, estradiol, GH, insulin, beta-endorphin), minerals (calcium, magnesium), binding protein (albumin), or blood lipids (TC, TG, HDL-C). Resting cardiovascular variables decreased similarly for both groups. These data suggest that 9 weeks of 500 mg/day gamma-oryzanol supplementation does not influence performance or related physiological parameters in moderately weight-trained males (Fry et al.1997).

TOXICOLOGICAL STUDIES ON RICE BRAN MAIN COMPONENTS

The enthusiasm for the use of rice bran oil in cardioprevention was initially clamped by an episode of alimentary rice oil contamination by a mixture of polychlorobiphenyls (PCB) in the Yu-Cheng village (central Taiwan) at the end of the 1970's (Chen et al.1984, Hirota et al.1993). The PCBs have been used in order to deodorise the oil during manufactur-

ing (Chen et al. 1985). At high temperatures, they transformed to polychlorodibenzofuran and polychloro-tetrphenyl able to produce a serious and peculiar chloracne on skin and mucous membrane. These symptoms, later named Yusho (oil disease), were often associated with much variable subjective symptoms and immunological abnormalities such as decreased concentration of IgM and IgA, decreased percentage of helper T-cells, suppression of delayed type response to recalling antigens and enhancement of lymphocyte spontaneous and enhanced proliferation (Asashi 1993, Lu et al.1985). The consequences of this mass poisoning of more than 2000 people are still studied after 20 years (Guo et al.1987, Soong et al.1997). This was the impulse that pushed researchers to investigate potential

cancerogenicity of rice bran oil and its main components. In 1987 Polasa and Rumini found evidence of the non-mutagenicity of rice bran oil using the bacterial reverse mutation Ames mutagenicity assay, with Salmonella typhimurium strains (Polasa et al.1987). Some year later, specific *in vitro* tests were carried out to confirm the short term safety of gamma-oryzanol. Its genotoxic and carcinogenic activity was studied in three genetic toxicity tests and the cancer promotion activity was studied in a cell-cell communication inhibitory test. gamma-oryzanol showed the negative response in the bacterial DNA repair test, bacterial reverse mutation tests and rat bone marrow chromosome aberration test. Also, it showed the negative response in the metabolic cooperation inhibition test using Chinese hamster V79 cells (Tsushimoto et al.1991). Moreover, ferulic acid has been found to inhibit azoxymethane-induced rat colon carcinogenesis when given in the post-initiation period (Tao et al.1997). Despite that a 1% gamma-oryzanol diet seemed to enhance rat lung carcinogenesis (Hirose et al.1991), the administration of a diet containing 2% gamma-oryzanol for 40 weeks to rats treated with 3,2'-dimethyl-4-aminobiphenyl did not modify the evolution of prostate atypical hyperplasias and carcinomas nor the incidences of different tumors in any other organs (Nakamura et al.1991). In 1992, Tamagawa et al. demonstrated *in vivo* non-toxicity and non-cancerogenicity of gamma-oryzanol in rats and mice. In these researches groups of 50 males and 50 females were fed a diet containing 0 (control), 200, 600 or 2000 mg gamma-oryzanol/kg body weight/day for 78 wk (mice) or 2 years (rats) and no significant treatment-related changes were observed in general condition, body weight, food consumption, mortality, organ weight or haematology nor in tumor incidence (Tamagawa et al.1992). Moreover, a more recent study of Yasukawa et al.

(1998) has demonstrated that rice bran oil active components (sitosterol ferulate, 24-methylcholesterol ferulate, cycloartenol ferulate and 24-methylenecycloartenol ferulate) markedly inhibited the 12-O-tetradecanoylphorbol-13-acetate (TPA) induced inflammation in mice. Furthermore, cycloartenol ferulate markedly inhibited the tumor-promoting effect of TPA in 7,12-dimethylbenz[a]anthracene-initiated mice. Finally, the modifying effects of gamma-oryzanol on the promotion stage of carcinogenesis were adequately investigated using several two stage carcinogenesis models in rats. In a multi-organ carcinogenesis model, male F344 rats were given combined treatment with different, and then treated with dietary 1% gamma-oryzanol acid or basal diet alone for 32 weeks. The incidence and multiplicity of lung tumors were slightly but significantly increased by high doses of gamma-oryzanol (100-150 times higher than the possible current human intake). Esophagus, colon, pancreas, kidney, bladder and thyroid lesion development was not influenced by this compound. Finally, examination of the modifying potential of gamma-oryzanol on mammary carcinogenesis in female Sprague Dawley rats pretreated with a single intragastric dose of 7,12-dimethylbenz(a)anthracene revealed no significant differences in the final incidences and multiplicities of mammary tumors, even if gamma-oryzanol tended to decrease the size of the tumor but without significant difference (Hirose et al.1999). The lung tumor promotion potential may not be due to gamma-oryzanol's major metabolite, ferulic acid, since ferulic acid at a dietary dose of 2% did not show such enhancing effect in Sprague-Dawley female rats pretreated with 1,2-dimethylhydrazine and N-methylnitrosourea (Hirose et al.1994). Moreover, the incidence of tongue carcinomas and preneoplastic lesions in rats given ferulic acid in the diet at a dose of 500 ppm after 5 week exposure to 20 ppm 4-nitroquinoline-1-oxide (4NQO) in drinking water was significantly lower on termination of the experiment (32 weeks) in comparison with a group treated only with 4NQO (Mori 1999). Regarding the other rice bran oil components, phytosterols are poorly absorbed and efficiently eliminated via the biliary route. No relevant side effects were observed in adult humans as well as children treated with high phytosterol doses (Becker et al.1993, Weststrate et al.1998).

CONCLUSIONS

A computer search on MedLine and Embase using the search terms "rice bran", "gamma-oryzanol", and "tocotrienol" (period: from 1966 to 2004), leads to the discovery of almost 150 non-matched referred articles about their supposed therapeutic (and toxic) properties. This is very indicative of the high level of interest in rice bran derived products. However, these studies did not produce unequivocal conclusions and have been run on animal species very different from each other, and using various methodologies and different targets. gamma-oryzanol is already commercialised for cosmetic use, as demulcent, restructuring and antioxidants to skin lips and hair. It is not completely clear what its real utility is, in this field. Even if gamma-oryzanol has been proposed on the

international market as an anabolic natural substance of use to sporting people, Fry's 1997 study disproved this kind of effect (Fry et al.1997). More promising studies appraise the ability of the oil to reduce by about 40% TC and LDL-C plasma levels and at the same time increasing HDL-C (Sugano et al.1997, Rhaguram et al.1995). On the basis of our clinical experience, we believe that rice bran oil could reduce by about 20% toward TC, LDL-C and TG, but does not significantly increase HDL-C. However, these results would be very satisfactory to most moderate hyperlipoproteinemic patients. Once the antihyperlipidemic properties of rice oil are confirmed, our aim is to define whether or not this agent has the fundamental characteristics to be utilised in cardiovascular prevention (Table 3). Available studies provide some assurance about non-toxicity and non-carcinogenicity of rice bran oil and its main components (de Deckere et al.1996). Moreover they could have a positive effect on peptic disease (gamma-oryzanol) (Ichimaru et al.1984), contribute to lower, the risk for colon cancer and benign prostatic hypertrophy (phytosterols) (Nair et al.1984, Berges et al.1995) and modulate allergic reactions (ferulic acid) (Hu et al.1991). The oil tastes pleasant (slightly sweet) and it should be suitable to fry food because of its high smoke point. This property is maybe related to the relatively high content in 4-monomethylsterols with an ethylidene side chain that may contribute to its oxidative stability (Kochhar et al.1983, White et al.1989). In European countries, it is now easily available both in pharmacy and supermarket and it is priced at the same level as extra-virgin olive oil. Beside the antihyperlipidemic action, the strong antioxidant property of gamma-oryzanol (Tajima et al.1983, Kim et al.1995, Hiramitsu et al.1991) and other rice bran oil components, such as tocotrienols and tocopherols may contribute to rice bran oil's antiatherogenic effect. However, a study of Schwab et al. showed that in middle-aged and elderly moderately hypercholesterolemic human subjects (age 44-78 y), the 32 day consumption of reduced-fat diets [30 energy percent (E%) fat, 17 E% protein and 53 E% carbohydrate] enriched in animal fat (beef tallow) or vegetable oils with a relatively wide range of fatty acid profiles (canola oil, corn oil, olive oil or rice bran oil) (20 E%) did not alter the *in vitro* susceptibility of LDL to oxidation (Schwab et al.1998). Moreover, of interest in the field of cardiovascular disease prevention, is the 1990's Seetharamaiah et al. study showing that gamma-oryzanol has a significant inhibiting power against ADP and collagen linked platelet aggregation in rats on a high cholesterol diet, but it did not alter this mechanism in rats on control diets (Seetharamaiah et al.1990). A very recent report also suggests that rice bran could be a relevant source of policosanols (Cravotto et al.2004), natural compounds able to inhibit platelet function as aspirin and the synthesis of cholesterol as low-dosed statins in humans (Varady et al. 2003). However, no one human study has been until now carried out with rice bran derived policosanols, but only with those derived from other natural sources.

In conclusions, wider randomised clinical trials would be useful to confirm the rice bran components reducing proper-

ties in plasma TC, LDL-C and TG and its capacity to raise HDL-C. Other important areas are the decrease of platelet aggregation and antioxidant action of rice bran components and above all the long-term-effect of a dietary supplementation with rice bran derived products on the main cardiovascular outcomes in human subjects. We suggest that rice bran oil could be considered an additional weapon in the management of mild to moderate hyperlipidemias.

CONFLICT OF INTEREST DISCLOSURE: None of the authors have any conflict of interest in the publication of this paper. At the time of submission we had no financial arrangement with any company whose product figures prominently in the submitted manuscript nor with a company making a competing product.

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