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## *Short Communication*

# Wild rice (*Zizania latifolia* (Griseb) Turcz) improves the serum lipid profile and antioxidant status of rats fed with a high fat/cholesterol diet

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The diet consumed by urban residents in modern China has become rich in saturated fats and cholesterol. In addition, the diet is high in carbohydrates from white rice and processed wheat starch. The aim of the present study was to determine the effects of replacing white rice and processed wheat starch with wild rice (WR) as the chief source of dietary carbohydrates. Rats fed with the diet patterned after the diet consumed by city residents of modern China showed elevated serum lipid levels comparable with rats consuming a high fat/cholesterol diet known to induce hyperlipidaemia in this species. Meanwhile, rats consuming the city diet with WR as the carbohydrate source suppressed the increase in serum TAG and total cholesterol, and the decrease in HDL cholesterol level. In addition, the rats fed the WR diet suppressed the build-up of oxidative stress by improving antioxidant capacity, increasing superoxide dismutase activity and reducing malondialdehyde concentration, both in the serum and liver. These findings illustrate that WR is effective in suppressing hyperlipidaemia and oxidative stress in rats even when the diet consumed is high in fat and cholesterol.

**Cholesterol: Hyperlipidaemia: Oxidative stress: Rats: Wild rice**

According to the first comprehensive Chinese survey in the fields of nutrition and health in 2002(1), the prevalence of adult overweight and obesity had increased by 39 and 97 %, respectively, over a 10-year period. As well, the prevalence rates of hypercholesterolaemia, hypertriglyceridaemia and low serum HDL cholesterol (HDL-C) were 2·9, 11·9 and 7·4 %, respectively, in Chinese adults. Statistical data indicated that the modern Chinese population exhibits excessive energy intake, poor choice of food types and decreased physical activity. A radical change in the composition of the diet ingested by Chinese residents, particularly among city dwellers, is the decreased intake of energy from cereals and protein per capita, and increased intake of energy from fat, especially from animal-derived foods(2). Clinical studies have demonstrated that adjustment of the dietary carbohydrate source to increase the dietary coarse grain, dietary fibre and constituent ratio of plant protein significantly improved body weight, BMI, lipid and glucose profiles(3–5), indicating that manipulation of dietary cereal composition can favourably improve syndromes associated with nutrition. Incorporation of novel grains into the diet may be a viable and effective strategy for achieving this goal.

There are four species of wild rice (WR) belonging to the genus *Zizania*. *Zizania aquatica* L., *Zizania palustris* L., and *Zizania texana* Hitche are native to North America, whereas *Zizania latifolia* (Griseb) Turcz is native to China, Japan and Vietnam. Little is known about the nutritional value of WR, but available studies indicate that WR has higher content of protein, dietary fibre, vitamin $B_1$, $B_2$, E and minerals than the common white rice(6–8). Chinese WR was once an important grain in ancient China, and was used as an herbal medicine to treat diabetes and other diseases associated with nutrition, but today its use for this purpose has disappeared. The present study was undertaken to determine whether intake of WR has desirable effects on serum lipid and antioxidant status in an animal model that is consuming a diet high in fat and cholesterol.

## Materials and methods

### *Animals and diets*

Male Sprague–Dawley rats (from SLAC laboratory animal company, Shanghai, China SCXK 2003-0003) were housed individually with a 12-h light–dark cycle and free access

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**Abbreviations:** CD, city diet; HFC, high fat/cholesterol; LF, low fat; MDA, malondialdehyde; SOD, superoxide dismutase; WR, wild rice.

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to water and a standard chow diet before the diet study. The present study followed the institutional and national guidelines for the care and use of animals, and all experimental procedures involving animals were approved by the Southeast University Animal Welfare Committee. The body weight of the rats was measured every 3 d. Rats (*n* 44) were acclimated by feeding the standard chow diet for 7 d, then divided into four groups (*n* 11) and fed one of the diets described in Table 1. The formulations of all diets meet the minimum requirement of the AIN-76A diet specification. The reference low fat (LF) diet is based on the AIN-76 formulation. The high fat/cholesterol (HFC) diet was used as a reference diet in the present study and is known to induce hyperlipidaemia in rats(9,10). The composition of the city diet (CD) is based on the diet consumed by urban residents in modern China, which is high in fat and cholesterol, and dietary carbohydrates are supplied mainly by white rice and processed wheat starch(2). For the WR diet, the white rice and processed wheat starch content were replaced with an equivalent amount of Chinese WR. The WR used in the study was collected from the Weishan lakes in Eastern China. The cultivated grains were confirmed to belong to the species *Z. latifolia* by the Nanjing Research Institute of Plants of the Chinese Academy of Sciences. The grains were sun dried, hulled and stored at 18–20°C before use. WR and white rice were milled into a powder sifted through a 1 mm screen and added directly to the experimental diet without pre-cooking. The white rice and processed wheat starch were bought from the local market. Since rats are resistant to diet-induced hypercholesterolaemia, cholesterol (1·5 % w/w) and porcine bile acids (0·2 % w/w) were added to the experimental diet to match that present in the reference HFC diet(9,10). In addition, egg yolk powder was added to increase the palatability of the experimental diets. During the experiment, all the rats were offered equivalent amounts of diet per day. To ensure the diet offered was completely consumed by each rat, the amount of food offered was 10–20 % below the amount of diets consumed *ad libitum* by rats of the same age in our preliminary studies. The amount of food offered was increased continuously from 6·0 g at the start of the experiment to 14·0 g at the end of the experiment. The feeding strategy employed ensured that all rats consumed equal feed rations daily within the duration of the experiment(11). Water was freely available from nipple drinkers. The duration of the experiment was 8 weeks.

**Table 1.** Composition (g/kg) of the diets

| Component | HFC | CD* | WR | LF |
|---|---|---|---|---|
| Casein | 215 | 215 | 215 | 230 |
| Sucrose | 265 | 0 | 0 | 310 |
| Maize starch | 258 | 0 | 0 | 295 |
| White starch† | 0 | 261 | 0 | 0 |
| White rice | 0 | 262 | 0 | 0 |
| Chinese wild rice | 0 | 0 | 523 | 0 |
| Cellulose | 50 | 50 | 50 | 50 |
| Lard | 100 | 100 | 100 | 0 |
| Bean oil | 0 | 0 | 0 | 70 |
| Egg yolk powder‡ | 50 | 50 | 50 | 0 |
| Cholesterol | 15 | 15 | 15 | 0 |
| Bile salts | 2 | 2 | 2 | 0 |
| AIN-76 vitamin mix | 10 | 10 | 10 | 10 |
| AIN-76 mineral mix | 30 | 30 | 30 | 30 |
| DL-Methionine | 3 | 3 | 3 | 3 |
| Choline chloride | 2 | 2 | 2 | 2 |
| Total carbohydrate | 537 | 448 | 436 | 611 |
| Total fat | 127·8 | 133·6 | 133·2 | 70·3 |
| Total cholesterol | 17·5 | 17·5 | 17·5 | 0 |
| Total protein | 233·9 | 280·2 | 300 | 233·5 |
| Total dietary fibre | 50·3 | 56 | 66 | 50·3 |
| Total energy (kJ/g) | 16·8 | 16·2 | 16·4 | 15·9 |
| Energy from fat (%) | 28·7 | 31·0 | 30·5 | 16·2 |
| Energy from carbohydrate (%) | 48·4 | 46·3 | 47·4 | 58·0 |
| Energy from protein (%) | 22·9 | 22·7 | 22·1 | 25·8 |

HFC, high fat/cholesterol; CD, city diet; WR, wild rice; LF, low fat.
* Patterned after the composition of the diet of city residents of modern China.
† Processed wheat starch.
‡ Contains 5 % (w/w) cholesterol.

### *Tissue sample management*

The rats were killed after 12 h of fasting. The weights of the liver and epididymal fat pads were recorded. Epididymal fat pad mass was used as an index of body fat mass(12,13). A portion of each liver was prepared for enzyme assays and histological measurements.

### *Biochemical analyses*

Blood samples were taken from the tail vein of fasted rats (12 h) at the start, 4th and 6th week of the diet study. At the end of the experiment, blood was collected from the abdominal aorta, and then centrifuged to separate serum. Total cholesterol, TAG and HDL-C levels in the serum were measured using commercial enzymatic kits (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China). The malondialdehyde (MDA) levels, superoxide dismutase activity (SOD) and antioxidant capacities of serum and liver homogenate were measured using commercial kits (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China). MDA assay uses thiobarbituric acid-reactive substances' concentration as a measure of lipid peroxidation and oxidative stress. The SOD assay was based on a previously described procedure(14). The antioxidant capacity assay measures the ability of antioxidants present in a serum and liver homogenate sample to inhibit the peroxidation of 2, 2′-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) by metmyoglobin in the presence of $H_2O_2$.

### *Statistical analysis*

Data were analysed by one-way ANOVA followed by the Tukey *post hoc* test using SPSS for Windows (v. 11.5; SPSS Inc., Chicago, IL, USA). In all analyses, the level of significance was set at $P < 0·05$.

## Results

### *Changes in body weight and organ/body weight ratios and liver histology*

At the beginning of the feeding period, the mean body weights (193·44 (SD 18·19) g) of the rats in all the groups were nearly identical. At the end of experiment, the mean body weight of rats fed with the HFC diet was significantly greater than that

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given the LF diet (399·03 (SD 17·17) and 349·42 (SD 17·99) g, respectively, $P<0·05$). The rats fed with the WR diet had lower body weight than the rats fed with the CD (361·22 (SD 20·83) and 392·28 (SD 19·32) g, respectively, $P<0·05$). The final mean body weight of the rats fed with the CD was comparable with that of rats fed with the HFC diet, but the final mean body weight of rats fed with the WR diet was comparable with that of rats fed with the LF diet. Furthermore, the HFC diet and the CD raised ($P<0·05$) the percent liver weight to body weight ratio (HFC, 3·32 (SD 0·18); CD, 3·28 (SD 0·23); WR, 2·77 (SD 0·36); LF, 2·52 (SD 0·21)) and percent epididymal fat weight to body weight ratio (HFC, 1·52 (SD 0·23); CD, 1·48 (SD 0·22); WR, 1·04 (SD 0·16); LF, 0·78 (SD 0·26)) relative to the WR and LF diets ($P<0·05$ in both cases). In the rats fed with the HFC diet, fatty degeneration of hepatocytes was obvious (data not shown). Hepatic cells were lipid filled and had incomplete nuclear membrane. Similar features were observed in the rats fed with the CD, but the degree of fatty degeneration of hepatocytes was less severe compared with the HFC diet group. These features were not prominent in the livers of rats fed with the WR diet. Fatty degeneration of hepatocytes was not obvious in the livers of rats fed with the LF diet, and the hepatocytes had complete and distinct nuclear membrane.

### *Serum lipid profiles and antioxidant status*

Compared with the WR diet, the CD increased the serum total cholesterol (Fig. 1(A)) and TAG (Fig. 1(B)), and decreased the serum HDL-C (HFC, 0·82 (0·09); CD, 0·99 (SD 0·09); WR, 1·34 (SD 0·06); LF, 1·40 (SD 0·10) mmol/l) level by 8th week ($P<0·05$). The CD induced similar increases in these parameters as did the HFC diet. The rats fed with the WR diet showed a serum lipid profile that was comparable with the rats fed with the LF diet. The rats fed with the LF diet had a lower serum MDA concentration than the rats fed with the HFC diet (Table 2, $P<0·05$). The serum MDA concentration of rats fed both with the HFC diet and CD was higher than that of the WR diet group ($P<0·05$). The serum MDA concentration of rats fed with the WR diet was comparable with that of rats fed with the LF diet ($P>0·05$). Furthermore, higher serum SOD activity and antioxidant capacity were verified in the LF diet group than the HFC diet group. Similarly, the WR diet group had higher serum SOD activity and antioxidant capacity than the CD group. The rats fed with the WR diet showed comparable serum SOD activity and antioxidant capacity with the rats fed with the LF diet. A similar pattern of changes were evident in the liver homogenates (data not shown).

## Discussion

The CD composition used in the present study was patterned after the diet of Chinese urban dwellers in modern China, and is high in fat, cholesterol and carbohydrates(2). The dietary carbohydrates are obtained mainly from white rice and processed wheat starch. In the present study, the CD was found to increase serum lipid and oxidative stress comparable with that found in rats fed the HFC diet, which is known to induce hyperlipidaemia in this species(9,10). The rats fed both with the CD and the HFC diet exhibited an increased TAG, total cholesterol and decreased HDL-C concentration and increased oxidative stress, characterised by decreased antioxidant capacity and SOD activity and increased MDA concentration. It should be noted that the antioxidant capacity reported may not fully represented due to the limitations of the method employed in the present study. Similarly, the specificity of the assay for MDA could be improved by using an HPLC-based method. Nevertheless, the results taken together indicate the increase in oxidative stress, consistent with the findings of studies on Chinese urban dwellers with hyperlipidaemia(1).

In the present study, we tested the hypothesis that changing the quality of dietary carbohydrates improves the serum lipid and oxidant stress status by replacing the dietary carbohydrate source of the CD with WR. We found that the WR diet exerted beneficial effects on the lipid profiles by preventing the increase in serum TAG and total cholesterol, as well as preventing the decrease in HDL-C level even when the diet remained high in saturated fats and cholesterol. This difference

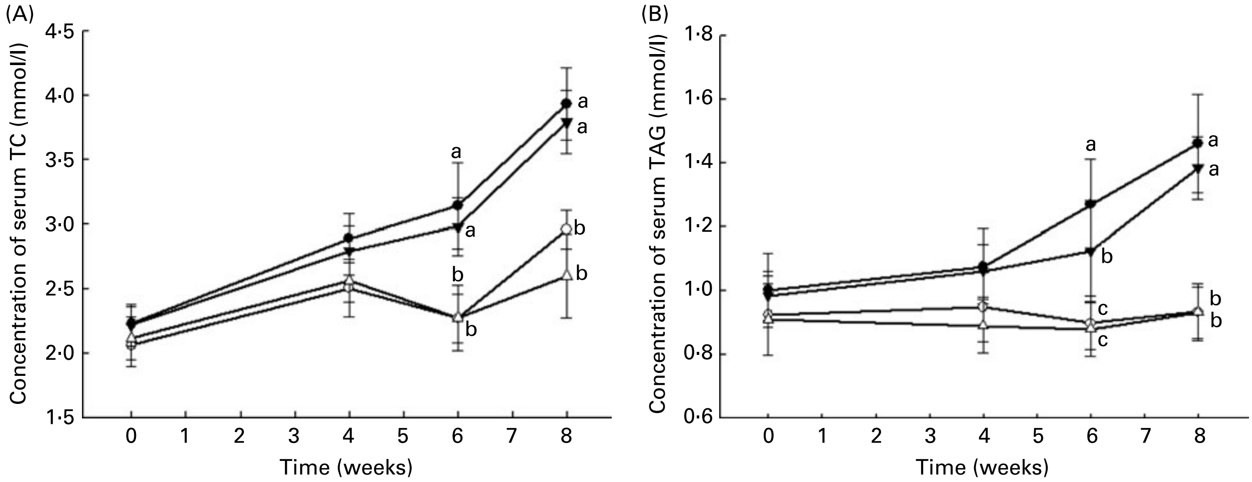

**Fig. 1.** The lipid profiles of rats (*n* 11) fed the experimental diets for 8 weeks. (A) Concentration of serum total cholesterol (TC) and (B) concentration of serum TAG. Values are means and standard deviations. [a,b,c]Values with unlike letters are significantly different ($P<0·05$; ANOVA followed by the Tukey *post hoc* test). -●-, High fat/cholesterol; -▼-, city diet; -○-, wild rice; -△-, low fat.

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**Table 2.** Oxidative stress index of serum from rats on experimental diets*

(Mean values and standard deviation for eleven rats per group)

| Diet group | MDA (mmol/l) |  | SOD (kU/l) |  | Antioxidant capacity (kU/l) |  |
|---|---|---|---|---|---|---|
|  | Mean | SD | Mean | SD | Mean | SD |
| HFC | 7·38$^{a}$ | 0·94 | 71·25$^{b}$ | 5·08 | 6·66$^{c}$ | 1·35 |
| CD | 4·36$^{b}$ | 0·51 | 76·97$^{b}$ | 8·46 | 11·81$^{b}$ | 1·73 |
| WR | 3·95$^{c}$ | 1·65 | 113·90$^{a}$ | 20·92 | 14·53$^{a}$ | 2·44 |
| LF | 3·83$^{c}$ | 0·83 | 105·69$^{a}$ | 13·84 | 14·87$^{a}$ | 2·70 |

MDA, malondialdehyde; SOD, superoxide dismutase; HFC, high fat/cholesterol; CD, city diet; WR, wild rice; LF, low fat.

[a,b,c] Mean values within a column with unlike superscripts are significantly different ($P < 0·05$; ANOVA followed by the Tukey *post hoc* test).

* See Table 1 for details on diet composition.

cannot be attributed to differential fat intake alone, since food intake throughout the feeding period was the same for rats that consumed the CD and the WR diet. Interestingly, the WR diet was also able to prevent excessive weight gain, despite similar dietary energy intake among the rats consuming the experiment diets in the study. In fact, rats fed the WR diet consumed slightly higher energy than the rats fed the CD over the course of the diet study (51·85 kJ per rat fed the WR diet *v.* 51·22 kJ per rat fed the CD).

The precise mechanisms for how WR improved serum lipid profiles in rats were not explored in the present study. The prevention of lipid-increasing action of the WR diet might be accounted for the unique nutrient composition of the WR. For example, WR has slightly higher plant protein content (the protein content of the WR diet was 30 *v.* 28 % in the CD), slightly lower amount of carbohydrate (the carbohydrate content of the WR diet was 43·55 *v.* 44·75 % in the CD) and higher content of dietary fibre than common white rice and wheat starch (the dietary fibre content of the WR diet was 6·6 *v.* 5·6 % in the CD)(6–8). Studies have shown that dietary protein origin and amount ingested can influence lipid and cholesterol metabolism, and compared with the animal protein, the plant protein, such as soyabean protein in general, produce lower plasma cholesterol levels(15–17). In addition, *Zizania* WR has a higher dietary fibre (3·4 % insoluble fibre and 0·79 % soluble fibre) content than white rice (0·34 % insoluble fibre and almost no soluble fibre) or wheat starch (1·5 % insoluble fibre and 1·5 % soluble fibre)(18,19). Previous studies showed that insoluble fibre is able to decrease food intake, gastric emptying, the rate and quantity of fat absorption, and influences fat oxidation and fat storage leading to lower postprandial glucose and lipid levels(20,21), and leads to decrease weight gain and improved insulin sensitivity(22). Soluble dietary fibres are fermented in the colon by intestinal microflora to SCFA such as acetate, butyrate and propionate, which have been associated with lowered plasma cholesterol and decreased risk of CVD(23). In the present study, the quality of protein and dietary fibres supplied by WR may be of greater importance since the quantity of these dietary components is similar in the CD and WR diets.

Several studies have suggested that oxidative stress is one of the causative factors of atherosclerosis(24,25). To counteract the oxidants, an important endogenous antioxidant system exists *in vivo*, which includes antioxidant compounds such as vitamin E and antioxidant enzymes such as SOD, CAT, GSH, GPx and GSH reductase(26). It has been reported that dietary supplementation of antioxidant substances such as vitamin E and some minerals such as Se and Zn can increase antioxidant capacity(27). The present study showed that the WR diet was able to inhibit the increase in oxidative stress, but the bioactive components responsible for the antioxidant effect were not uncovered. WR grain is rich in pigment, minerals and vitamins (vitamins $B_1$, $B_2$ and E)(6–8). Antocyanin pigments have antioxidant and free radical scavenging properties, and have been shown to increase resistance to lipid peroxidation in rats(28). Additional unidentified compounds with antioxidant properties may also be present in WR. Thus, the unique micronutrient composition of WR likely contributes to the favourable effects on antioxidant status that was observed in the present study. This possibility will be investigated in further studies.

In summary, we demonstrated in the present study that WR has desirable properties for improving serum lipid profiles and antioxidant status. Replacing white rice and wheat starch with WR appears to be an effective means of preventing hyperlipidaemia in rats even in a diet that is high in saturated fat and cholesterol.

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