

## Cooked Rice and Oat Meal Similarly Reduce Hypercholesterolemia in Rats

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### Abstract

Five groups of rats were given five different diets for 4 weeks after one week adaptation with standard casein. These diets include: casein diet as a control group (C), cooked rice (R), cooked rice with 1% exogenous cholesterol (RC), oat meal (O), and oat meal with 1% exogenous cholesterol (OC) (Different analysis was determined on animals of these groups at the end of the experiment). The rats weights at the end of experiment showed that weight gain of control group rats was significantly higher ( $p \leq 0.01$ ) than other groups but there were no differences among other groups.

The concentration of serum total cholesterol for (O) group (64.6 mg/100 ml) and that of (R) group (67.7 mg/100 ml) were significantly lower ( $p \leq 0.01$ ) than those of other groups and the control group was the highest one in all groups (112.42 mg/100 ml).

The results also showed significant differences among mean relative weights of Liver, spleen and heart of different groups. The highest and lowest values of mean relative weight of rat's liver, spleen and heart in experimental groups were : (O) group (3.87) and R group (3.29) for liver, (R) group (0.48) and (O) group (0.38) for spleen, both RC and R groups (0.37) and (O) group (0.33) for heart respectively.

The (R) and (O) diets significantly lowered the total fat content (24.0, 26.16 mg/kg respectively) while (O) diet lowered the cholesterol content (1.36 mg/kg) of the liver compared to the control diet (51.9 and 2.47 mg/kg for total fat and cholesterol respectively).

For bile acid content in the feces, there were significant differences ( $p \leq 0.01$ ) among the different groups; (OC) group showed the highest (10.4 mg/gm of feces) and the control group showed the lowest value (1.4 mg/gm of feces).

**الرز المطبوخ ووجبة الشوفان تخفضان مستوى الكولسترول في الفئران بشكل متشابه**

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### الخلاصة

خمسة مجاميع من الفئران تمت تغذيتها على خمسة أنواع من العلائق ولمدة أربعة أسابيع وذلك بعد أسبوع واحد من التأقلم على الكازين القياسي. وهذه العلائق تتضمن: الكازين ولحده لمجموعة السيطرة (C)، الرز المطبوخ (R)، الرز المطبوخ مع 1% كولسترول خارجي (RC)، وجبة الشوفان (O)، وجبة الشوفان مع 1% كولسترول خارجي (OC) وقد تمت التحاليل المختلفة على الحيوانات لهذه المجاميع بعد انتهاء فترة التجربة. أظهرت مجموعة السيطرة ارتفاعا في وزن الفئران وكانت أعلى من المجاميع الأخرى ( $p \leq 0.01$ ) في نهاية فترة التجربة ولم تكن هناك فروقات معنوية بين المجاميع المختلفة الأخرى.

كان تركيز الكوليسترول في سيري الدم لفئران مجموعة (O) (64.6 ملغم / 100 مل) ولمجموعة (R) (67.7 ملغم / 100 مل) وهاتان المجموعتان هما اقل مستوى ( $p \leq 0.01$ ) من المجموع الأخرى في حين كانت مجموعة السيطرة هي الأعلى (112.42 ملغم / مل) بين المجموع. وقد أظهرت النتائج اختلافات معنوية بين معدلات أوزان الكبد والطحال والقلب للمجموع المختلفة. وكانت أعلى وزن وأقل وزن للكبد والطحال والقلب للمجموع هي 3.87 غم و 3.29 غم لمجموعة (O) و (R) على التوالي بالنسبة للكبد و 0.48 غم و 0.38 غم للمجموعتين (R) و (O) بالنسبة للطحال على التوالي أما بالنسبة للقلب فقد كانت المجموعتين (R) و (RC) 0.37 وكانت مجموعة (O) 0.33 غم.

وقد خفضت العليقتين الخاصة بالمجموعتين (R) و (O) من المحتوى الدهني للكبد بشكل معنوي (24.00 و 26.2 ملغم / كغم على التوالي). وخفضت عليقة مجموعة (O) محتوى الكوليسترول في الكبد (1.36 ملغم / كغم) مقارنة مع مجموعة السيطرة (51.9 و 2.47 ملغم / كغم) للدهن الكلي والكوليسترول على التوالي. أما بالنسبة للحامض الصفراوي في البراز فقد ظهرت اختلافات معنوية ( $p \leq 0.01$ ) بين جميع المجموع. وقد كانت مجموعة (OC) هي الأعلى (10.429 ملغم / غم من البراز) وكانت مجموعة السيطرة هي الأقل (1.434 ملغم / غم من البراز).

## Introduction

The composition of our diet plays an important role in the management of lipid and lipoprotein concentrations in the blood. In recent years, however, the possible hypocholesterolemic effects of certain diets and dietary components, such as rice and rice protein (1, 2, and 3) tocopherol (4 and 5) and  $\beta$ -glucan (4, 6, and 7) have been investigated.

The treatment of cardiovascular disease with rice diet was suggested several years ago (2). However, there are different types of rice. The most common rice consumed by people is the white rice; the rest is red and black rice. The hypocholesterolemic effect of rice protein diet may be due to its relative low content of methionine as indicated by Morita et al. (3).

Dietary fiber and its effect on cholesterol and cardiovascular diseases have been widely studied in the last decade.

Dietary fibers can be divided in two major groups, water soluble (Pectin, gums, mucilages, and some hemicelluloses), and water insoluble (lignins, cellulose and the remainder of the hemicelluloses) (8). Water-soluble fiber in the diet such as oats, and psyllium was shown to be an additional important component of cholesterol reduction factor (6, 8, 9, 10, and 11). In contrast, water insoluble fiber such as wheat fiber and cellulose do not lower serum cholesterol levels (11).

The present study was designed to investigate the influence of cooked rice and oat diets on total plasma cholesterol with and without addition of 1% cholesterol, in addition to show their effects on cholesterol and fat in the liver and feces.

## Materials and Methods

### Animals:

Male rats were obtained from the National Center of Pharmaceutical Research, Ministry of Health, Iraq. The rats were distributed into 5 groups and were housed in wire-bottomed cages in controlled room temperature ( $25 \pm 2^\circ\text{C}$ ). Animals had free

access to standard casein diet as used by Morita et al.(3) and water for one week before the experiment was started.

The five different diets that were fed in this study include: casein diet (C), cooked rice diet (R), cooked rice with 1% exogenous cholesterol diet (RC), oat meal diet (O), and oat meal diet with 1% exogenous cholesterol (OC) for 4 weeks after one week adaptation period with the casein diet. The same amounts of vitamins and minerals were added to all these diets and the body weight and food consumption of rats were measured (see tables 1 and 2).

**Biochemical Analysis:**

Blood was collected by heart puncture and transferred to plastic tubes containing 100µ of heparin. Plasma was separated from whole blood by centrifugation at 200 Xg for 20 minutes and was stored at 4°C until subsequent analysis of plasma cholesterol concentrations as reported by Franey and Amandor (12).

At the end of experiment, one rat from each group was deprived of food overnight and killed under ether anesthesia. The liver, spleen and heart of rats were harvested, washed with isotonic saline and weighted. Tissue samples were stored at -20°C until used for analysis for fat and cholesterol concentration as mentioned by Folch et al.(13). Bile acids in the feces were determined using Thin Layer Chromatography (14).

**Statistical analysis:**

Results were expressed as means for animals in each diet group. The differences among the five dietary groups were tested by the analysis of variance using SAS software. The differences among groups were compared by Duncan’s multiple range test and the differences of  $p \leq 0.01$  were regarded as significant.

**Results**

The body weight gain in the control group was significantly higher than those of other diets, while the mean food intake of (R) and (RC) groups were significantly higher than the others (Table 2). The serum total cholesterol of control rats (112.4 mg/100 ml) was significantly higher than other dietary groups. Also in rats fed 1% cholesterol (OC and RC group), the serum total cholesterol concentrations (78.3 and 80.8 mg/100ml respectively) were significantly ( $p \leq 0.01$ ) greater than (O) and R groups (64.60 and 67.7mg/100m) as it is shown in Table (3).

There were significant differences ( $p \leq 0.01$ ) between mean relative weights of liver (3.87, 3.29), spleen (0.38, 0.48), and heart (0.33, 0.37) of rats fed the (O) and (R) diets (Table 4). The same table shows that liver mean relative weight of rats fed 1% cholesterol (OC, RC groups) are compatible to those of (C) group (3.83 and 3.48 for OC and RC groups compared to 3.6 for (C) group). Table (5) revealed that feeding rats the (O) and (R) diets significantly ( $p \leq 0.01$ ) lowered the concentration of liver and fecal total fat compared to the rats fed (C), (OC), and (RC) diets. The mean concentration of liver cholesterol was significantly ( $p \leq 0.01$ ) lower in rats fed the (O) diet compared to other groups. Conversely, the fecal cholesterol level in rats was significantly ( $p \leq 0.01$ ) affected by (OC) and (RC) diets. And consequently, significantly higher amount of fecal bile salts were excreted by rats fed (OC) and (RC) diets.

**Table (1) Composition of casein standard diet that fed to the rat**

Component	g/Kg diet
Casein	250
Starch	535
Sucrose	100

Sunflower oil	70
Mineral mixture	35
Vitamins mixture	10
Total	1000 gm

**Table (1-b) composition of mineral mixture that used in standard diet**

Type of mineral	Structure	Quantity (gm)
Calcium phosphate	CaHPO <sub>4</sub>	500
Sodium chloride	NaCl	47
Potassium citrate Monohydrate	K <sub>3</sub> C <sub>6</sub> H <sub>5</sub> O <sub>7</sub> . H <sub>2</sub> O	22
Potassium Sulphate	K <sub>2</sub> SO <sub>4</sub>	52
Magnesium Oxide	MgO	24
Manganous Carbonate	43 – 48% Mn	3.5
Ferric Citrate	16 – 17% Fe	6
Cupric Carbonate	53 – 55% Cu	0.3
Potassium Iodide	KIO <sub>3</sub>	0.01
Sodium Selenite	Na <sub>2</sub> SeO <sub>3</sub> . 5H <sub>2</sub> O	0.01
Chromium Potassium Sulphate	CrKSO <sub>4</sub> .12H <sub>2</sub> O	0.55
Add Sucrose to make 1000 gm		

**Table (1c) composition of vitamin mixture that used in standard diet**

Mix	Each 1 kg contain
Vitamin , acetate	800,000 IU
Vit D3	150,000 I.U.
Vit E	1000 mg
Vit B1	500 mg
Vit B2	500 mg
Calcium – d- pantothenate	4,000 mg
Vit B6	200 mg
Vit B12	8 mg
Folic acid	50 mg
Vit K3	2000 mg
Nicotinic acid	6000 mg
Iron sulphate	0.5 gm
Manganese Sulphate	0.4 gm
Cobalt Chloride	Chloride 0.01 gm
Zinc Sulphate	0.15 gm
Copper Sulphate	0.04 gm

**Table (2) The increase in Body weight, Feed intake and Feed efficiency of rats in the experimental groups for 28 days**

Diet Group	Initial weight (g)	Final weight (g)	Increase in weight	food intake (g/28day)
Control (C)	85.0	148.1 <sup>a</sup>	63.1 <sup>a</sup>	335.0 <sup>b</sup>
Oat meal (O)	87.8	126.4 <sup>b</sup>	38.6 <sup>b</sup>	265.5 <sup>b</sup>
Oat meal + 1% cholesterol (OC)	84.5	134.7 <sup>ab</sup>	50.1 <sup>ab</sup>	312.0 <sup>b</sup>
Rice (R)	81.0	123.5 <sup>c</sup>	42.5 <sup>b</sup>	639.8 <sup>a</sup>
Rice + 1% cholesterol (RC)	89.9	130.5 <sup>ab</sup>	40.6 <sup>b</sup>	648.9 <sup>a</sup>

\* Values bearing different letters in a column are significantly different (p<0.01).

**Table (3) Change in serum cholesterol level after one week on standard diet and 28 on different diet groups**

Diet Groups	Before treatment Mg/100 ml	After one week on standard diet Mg/100 ml	After 28 days on experimental diets mg/100ml
Control (C)	63.0	70.8	112.4 <sup>a</sup>
Oat meal (O)	63.0	73.5	64.6 <sup>c</sup>
Oat meal + 1%	60.4	71.9	78.3 <sup>b</sup>

cholesterol (OC)			
Rice (R)	63.6	72.7	67.7 <sup>c</sup>
Rice + 1% cholesterol (RC)	59.4	74.2	80.8 <sup>b</sup>

\*Values of different letters in a column are significantly different (p<0.01)

**Table (4) Weights of organs (liver, spleen, heart), and its relative weight (organ weight/body weight) in different diet groups at the end of experiment**

Group	Liver		Spleen		Heart	
	Weight (g)	Relative weight	Weight (g)	Relative weight	Weight (g)	Relative weight
Control (C)	5.46 <sup>a</sup>	3.60 <sup>bc</sup>	0.70 <sup>a</sup>	0.47 <sup>a</sup>	0.51 <sup>a</sup>	0.34 <sup>b</sup>
Oat meal (O)	4.84 <sup>c</sup>	3.87 <sup>a</sup>	0.50 <sup>c</sup>	0.38 <sup>b</sup>	0.42 <sup>c</sup>	0.33 <sup>b</sup>
Oat mal + 1% cholesterol (OC)	5.10 <sup>b</sup>	3.83 <sup>ab</sup>	0.57 <sup>b</sup>	0.42 <sup>b</sup>	0.47 <sup>b</sup>	0.34 <sup>b</sup>
Rice (R)	4.08 <sup>e</sup>	3.29 <sup>d</sup>	0.60 <sup>b</sup>	0.48 <sup>a</sup>	0.46 <sup>b</sup>	0.37 <sup>a</sup>
Rice + 1% cholesterol (RC)	4.46 <sup>d</sup>	3.48 <sup>c</sup>	0.56 <sup>b</sup>	0.42 <sup>b</sup>	0.49 <sup>ab</sup>	0.37 <sup>a</sup>

\* Values with different letters are significantly different (p<0.01)

**Table (5) Total fat, cholesterol in liver and total fat, cholesterol and bile acids in feces at the end of the experiment**

Diet groups	Liver		Feces		
	Total Fat (mg/Kg)	Cholesterol (mg/Kg)	Total Fat (mg/Kg)	Cholesterol (mg/Kg)	Bile acids (mg/g feces)
Control (C)	51.9 <sup>b</sup>	2.47 <sup>a</sup>	96.8 <sup>a</sup>	5.85 <sup>b</sup>	1.434 <sup>e</sup>
Oat meal (O)	26.2 <sup>c</sup>	1.36 <sup>c</sup>	36.6 <sup>c</sup>	4.25 <sup>b</sup>	3.636 <sup>d</sup>
Oal mal + 1% cholesterol (OC)	57.4 <sup>b</sup>	1.86 <sup>b</sup>	58.4 <sup>b</sup>	21.76 <sup>a</sup>	10.429 <sup>a</sup>
Rice (R)	24.0 <sup>c</sup>	1.76 <sup>b</sup>	41.0 <sup>c</sup>	7.54 <sup>b</sup>	4.586 <sup>c</sup>
Rice + 1% cholesterol (RC)	91.0 <sup>a</sup>	2.51 <sup>a</sup>	55.9 <sup>b</sup>	29.05 <sup>a</sup>	9.926 <sup>b</sup>

\* Values with different letters are significantly different (p<0.01)

## Discussion

In 1997, the US Food and Drug Administration passed unique rule that allowed oat bran to be registered as a first cholesterol-reducing food (7).

The composition of the human diet plays an important role in the management of lipid and lipoprotein concentrations in blood (1, 15, and 16). Reduction in cholesterol intake has been traditionally the first goal of dietary therapy in lowering the risk of cardiovascular diseases (4). Cholesterol metabolism is determined by diet, genetics, cholesterol absorption and sterol synthesis and excretion (17). Many years ago, much attention has been given to the role of dietary metabolism (4).

Although the total fiber concentration of rice is low (18) in rats consuming cooked rice diet (R) or cooked rice with cholesterol (RC), rice had dramatically reduced the total serum cholesterol compared to control (C group) diet (Table 3). This result agreed with previous studies in human (9 and 11) and rats (1, 3, and 19) who revealed that ingestion of plant proteins lowered total cholesterol concentration more than the animal protein did. The explanation of this effect could be related to differences between plant and animal proteins in their amino acids contents.

Sugiyama et al. (20) indicated that the decrease in the ratio of phosphatidylcholine (PC) to phosphatidyl ethanolamine (PE) in the microsomal phospholipids profile may be associated with the hypocholesterolemic action of some plant proteins.

Methionine has a hypercholesterolemic action when added to cholesterol free diets in rats (16), and a significant positive correlation has been observed between plasma cholesterol concentration and the methionine content of protein (20). Rice meal effects in reducing plasma cholesterol in this study could be related to low methionine level of rice protein which agreed with what found by Morita et al.(3).

As shown in table 3 of this study, the oat diet reduced serum total cholesterol. Numerous studies demonstrated that whole grains that are high in soluble fiber, such as oats, are more effective in lowering blood cholesterol than other type of grains in which the fiber is insoluble such as wheat (8 and 15).

Several mechanisms has been suggested regarding soluble fiber action including: affecting excretion of bile acids (3, 6, 11, and 21), increased viscosity of the intestinal contents which interferes with micelle formation (11) and the soluble fiber responsible for formation of thick unstirred water layer adjacent to the mucosa that acts as a physical barrier in reducing the absorption of nutrients and bile salt (4). Production of short-chain fatty acids, such as acetate and propionate after fermentation of soluble fibers by bacteria may inhibits hepatic cholesterol synthesis is another new mechanism about the fiber action in reducing blood cholesterol (4, 15).

It has been shown by many investigators that the primary bile acids are synthesized from cholesterol in the liver, and cholesterol 7- $\alpha$ -hydroxylase is considered to catalyze the rate limiting step in the biosynthesis process. Hepatic bile acid synthesis is controlled in the liver through negative and positive feedback mechanisms. Bile salts synthesis by the liver is controlled and regulated by 7- $\alpha$ -hydroxylase activity (22, 23, 24, and 25). Thus, increased bile salts excretion out of the body (Table 5) may promotes bile salts synthesis from cholesterol through decreased feedback inhibition of cholesterol 7- $\alpha$ -hydroxylase by bile salts (17, 26).

From the results of present study, we conclude that consumption of cooked rice or oat meals that result in significant serum total cholesterol reduction could be caused by fecal bile salts attachment to the fiber in rats gut. These results indicate that addition of fiber source to a healthy diet could help in reducing the risk of cardiovascular diseases.

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