

Effect of Addition of Methionine to Rice Protein on the Cholesterol and Lipoproteins Concentrations in Rats

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ABSTRACT

The effect of the addition of methionine to rice protein on the levels of plasma and lipoproteins cholesterol in relation to the casein were investigated in rats. Rats were fed diets containing casein (C), rice protein (RP), casein and colfibrate (CC), and rice protein with methionine (RPM). Serum total cholesterol concentrations were lower in rats fed RP, CC, and RPM diets than those fed C diet. The concentration of liver cholesterol was also lower in rats fed the RP diet.

The serum high density lipoprotein (HDL) cholesterol level and fecal bile acids were higher in rats fed (RP) diet than in those fed (C) or RPM diets.

These results indicated that increased fecal bile acids excretion may be responsible for the antihypercholesterolemic effect of rice protein compared with casein.

Introduction:

Coronary heart diseases (CHD) are major health crisis in developed and developing countries. The elevated concentration of total or LDL cholesterol in the blood are powerful risk factors for CHD, whereas high concentration of HDL cholesterol or a low LDL to HDL cholesterol ratio may protect against CHD (1 and 2). Consumption of diets high in whole grains has been reported to have beneficial health effects such as a reducing risk of cancer, cardiovascular diseases and blood cholesterol. These results have been ascribed to have effects of the fiber content of whole grain foods on risk factors for these diseases (3). American Heart Association suggests that the ratio of carbohydrates in the diet should be increased, especially by taking food rich in complex carbohydrates.

Rice is the staple food in many Asian countries and also the main source of carbohydrates. Although the total fiber concentration in polished rice is low, polished rice has been revealed to lower serum cholesterol (1), and that the hypocholesterolemic action of plant proteins is due to the low methionine content of that proteins (4).

The treatment of cardiovascular diseases with rice diet was suggested several decades ago. More than 50 years ago, it was stated that consumption of white rice decreased blood pressure and lowered hypercholesteremia in humans (5). The current study was designed to examine the effect of rice proteins in lowering cholesterol concentration in rats, and if the addition of methionine to a rice protein could eliminate the hypocholesterolemic effects of rice protein compared to casein effect.

Materials and Methods:

Animals:

Male rats, weighing 100gm±5 were obtained from medical school, Al-Nahrain University, Baghdad-Iraq. The rats were housed in screen-bottomed, stainless steel cages in a room maintained at 25±1°C. Animals were divided into four groups on the basis of body weight. Each group was fed freely for four weeks. The test diets were casein (C), rice protein (RP), rice protein with 0.22% methionine (RPM), and casein with 10mg colfibrat (CC) per day. The composition of each test diet was the same as the casein-starch diet except for protein source (table 1).

Proteins:

A crude rice protein fraction was extracted from

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rice as described by Morita et al. (6). This fraction contained 86% protein, 4% carbohydrate, and about 9% moisture.

Body weight and food intake were recorded each morning before replenishing the diet. At the end of the experiment, all rats were deprived of food overnight and killed under diethyl ether as anesthesia.

Collection of Blood Samples:

Blood was collected from each rat under chloroform anesthesia via heart puncture and transferred into sample tubes containing EDTA as an anticoagulant (7). The serum from each blood sample was recovered by centrifugation at 3000 rpm and stored at -20°C.

Biochemical Analysis:

Serum total cholesterol, and HDL-cholesterol concentrations, and the LDL-cholesterol concentrations were estimated using Biometriex (France) kit. The LDL-cholesterol and very low density lipoprotein in the serum were obtained by subtracting the value of HDL-cholesterol from the total cholesterol as mentioned earlier (7). The total cholesterol in some organs was measured according to Franey and Elias (8). Total fecal bile salts were determined according to the method of Kritchevsky et al. (9) using Thin-Layer Chromatography (TLC).

Statistical Analysis:

All data were expressed as means for animals in each diet group. The statistical significance of mean differences between dietary groups was tested by the ANOVA. Significant differences among means were tested by Duncan's. A 1% level of probability was used to define differences as significant.

Results:

The body weight gain, food intake and food efficiency of the rats at the end of the four weeks are shown in Table 2. The body weights of the rats before the experiment were 95-101gm compared to 133-229gm at the end of the experiment. There were significant differences ($p \leq 0.01$) in final body weights among rats fed the various diets. All the rats had significantly less food intake compared to the animals of (CC) group (Table 2).

Animals fed on the casein diet showed total serum cholesterol, HDL cholesterol, and LDL+VLDL

cholesterol of 127.40, 21.50, and 105.89mg/100ml respectively. Serum total cholesterol concentrations (Table 3) in rats fed the RP, CC, and RPM diets (which were 51.78, 60.83, and 87.00mg/100ml respectively) were significantly ($p \leq 0.01$) less than in rats fed casein diet (127.40mg/100ml) However, the serum HDL cholesterol level decreased significantly ($p \leq 0.01$) in rats fed RPM and casein diets (17.90, 21.50mg/100ml respectively), while LDL+VLDL cholesterol levels increased significantly ($p \leq 0.01$) in rats fed RP diet (23.59mg/100ml) as well as the CC (26.07mg/100ml), and RPM diets (69.08mg/100ml) in comparison with those fed casein diet (105.89mg/100ml).

Feeding the RP diet significantly decreased the concentration of liver total lipids (6.88) relative to the casein diet (9.78), the CC diet (9.44), and the RPM (9.18), but had no significant effect on spleen or heart total lipid (Table 4). The mean concentrations of liver cholesterol significantly ($p \leq 0.01$) decreased in rats fed the RP diet (12.95) compared to those fed casein diet (13.86), CC diet (13.65), and RPM diet (13.55 mg/gm).

The mean concentration of spleen and heart cholesterol decreased significantly ($p \leq 0.01$) in rats fed RPM compared to those fed RP and CC diet, but had no effect on rats fed casein diet (Table 5).

Fecal total fat content in rats fed RP diet was significantly ($p \leq 0.01$) higher than that of rats fed the casein, CC, and RPM diets (Table 5). Significantly higher ($p \leq 0.01$) amount of fecal cholesterol (28.92mg) and total bile acids (5.08mg) were extracted from rats fed RP compared to rats fed casein (15.4mg and 1.18mg) and RPM (15.26mg and 1.5mg) diets.

Discussion:

Cholesterol and LDL are important lipids playing a major role in cardiovascular diseases (7). This has led to search for specific food components that may help to reduce the harmful effect of hyperlipidemia.

After four weeks, this study showed that rats fed RP had significant ($p \leq 0.01$) effect on total serum, HDL, and LDL+VLDL cholesterol compared to rats fed casein or RPM diets.

There were no significant differences in total and VLDL+LDL cholesterol in animals which consumed RP and CC diets (Table 3). A number of

previous studies in humans and animals revealed that rice consumption decreased total, and VLDL+LDL cholesterol and increased HDL cholesterol (5, 6, 10, 11, and 12). This hypercholesterolemic effect of RP diet and increased HDL cholesterol level (or HDL/VLDL+LDL ratio) may be due to its relatively low content of methionine as indicated by Morita et al. (6).

Methionine was shown to elevate serum cholesterol concentration (4). However, methionine addition to a rice protein diet (RPM) did not eliminate the hypocholesterolemic effects of RP relative to casein (Table 3), signifying that some factors other than methionine may be accountable at least in part for the cholesterol-lowering effect of rice protein. It was reported that the higher ratio of methionine to glycine in casein may be responsible for elevating the serum cholesterol (6) and glycine addition to a casein-based diet lowered the serum cholesterol level in rats (4).

As with other researchers (6), we do not have clear mechanism about the action of methionine on cholesterol metabolism. Oda et al. (13) has shown that the soybean protein diet lowered the expression of hepatic apolipoprotein A-1 mRNA and led to a decrease of apolipoprotein A-1 and HDL secretion from liver in the rats. However, whether some components in rice such as methionine is responsible for the increase of HDL level or not requires further studies. Sugiyama et al. (4) supported a possibility that the plasma cholesterol-elevating efficacy of methionine is attributable to its methyl group.

This study showed that both fats and cholesterol levels in liver, spleen, and heart decreased in rats fed RP diet compared to those fed (C) diet. In fact, others also have noted that the level of liver cholesterol was lower in rats fed RP diet (6) and in rats fed the plant protein (14).

Feeding rats RP diet enhanced fecal bile acid, cholesterol and fat excretion (Table 5). Moundrase et al. (15) suggested that a 40-50% rise in bile acid excretion is needed to considerably depress plasma cholesterol and stimulated hydroxymethyl glutaryl CoA reductase and cholesterol 7- α -hydroxylase.

In conclusion, the decreased intestinal absorption of cholesterol, and increased fecal bile salts excretion are primarily responsible for hypocholesterolemic effect of rice protein compared to casein diet.

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Table (1): Composition of Rats diet

Component	g/Kg diet
Casein	250
Starch	535
Sucrose	100
Sunflower oil	70
Mineral mixture	35
Vitamins mixture	10

Table (2): Body weight, Feed intake and Feed efficiency of rats in experimental groups

Diet Group	Initial weight (g)	Final weight (g)	Body weight (g/28 day)	food intake (g/28 day)	% Feed efficiency
Casein (C)	101.69 a	219.48 a	117.80 b	302.9 9 b	38.38 a
Rice (RP)	99.20 a	159.39 bc	60.01 cd	284.4 5 b	21.05 cd
Casein + colfibrate (CC)	100.25 a	229.10 a	129.07 a	450.0 6 a	28.67 ab
Rice + methionine (RPM)	95.0 a	133.33 c	38.33 d	289.6 2 b	13.19 d

* Values bearing different letters are significantly different (p<0.01).

Table (3): Effect of experimental diets of different protein on serum total, HDL, and LDL+VLDL cholesterol.

Diet Groups	Before treatment			After one week on Casein diet			After 4 weeks on experimental diets		
	T.C.	HDL	LDL+VLDL	T.C.	HDL	LDL+VLDL	T.C.	HDL	LDL+VLDL
	mg/100ml			mg/100ml			mg/100ml		
Casein (c)	69.61 ab	20.55 bc	49.06 b	92.16 a	24.24 c	67.92 ab	127.4 a	21.50 d	105.89 a
Rice Protein (RP)	68.97 ab	18.82 Abc	50.43 b	91.62 a	21.42 d	70.20 a	51.78 d	28.18 c	23.59 d
Casein + Colfibrate (CC)	69.29 ab	25.15ab	44.11 b	88.53 a	27.26 bc	61.26 c	60.83 d	34.75 a	26.07 d
Rice + Methionine (RPM)	61.28 b	27.62 a	33.65 c	78.87 a	29.81 a	49.05 d	87.0 c	17.90 e	69.08 bc

* Values bearing different letters are significantly different (p<0.01).

Table (4): Total fat and cholesterol in some organs of rats in the experimental groups at the end of four weeks.

	Total Fat			Cholesterol		
	Liver (mg/g)	Spleen (mg/g)	Heart (mg/g)	Liver (mg/g)	Spleen (mg/g)	Heart (mg/g)
Casein (c)	9.78 a	6.46 a	10.7 a	13.86 a	8.84 a	13.84 a
Rice Protein (RP)	6.88 da	6.15 a	9.65 a	12.95 c	8.48 bc	10.61 b
Casein + Colfibrate (CC)	9.44 a	6.23 a	9.90 a	13.65 ab	8.24 cd	9.22 c
Rice + Methionine (RPM)	9.18 ab	6.80 a	10.1 a	13.55 ab	8.91 a	13.61 a

* Values bearing different letters are significantly different (p<0.01).

Table (5): Total fat, cholesterol and bile acids in rats feces in experimental groups at end of four weeks.

Diet groups	Total Fat (mg/g)	Cholesterol (mg/g)	Bile acids (mg/g)
Casein (c)	70.1 d	15.4 c	1.18 d
Rice Protein (RP)	107.3 a	28.92 a	5.08 a
Casein + Colfibrate	96.4 b	25.62 a	3.56 b

(CC)			
Rice + Methionine (RPM)	74.4 d	15.26 c	1.53c d

* Values bearing different letters are significantly different ($p < 0.01$).

اضافة الميثيونين الى بروتينات الرز وتأثيرها على تركيز الكولسترول واللايبوبروتينات في الجرذان

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الخلاصة:

تم فحص تأثير اضافة الميثيونين الى بروتينات الرز على مستوى اللايبوبروتينات في بلازما الدم مقارنة مع تأثير الكازين في الجرذان. غذيت الجرذان بعليقة من الكازين (C)، بروتينات الرز (RP)، كازين وكولي فابريت (CC)، بروتينات الرز مع الميثيونين (RPM). انخفض تركيز الكولسترول لمصل الدم في الجرذان المغذاة بالعلائق (RP)، (CC) و (RPM) لكثير من الفئران المغذاة بالعليقة (C)، وان تركيز الكولسترول في كبد الجرذان كذلك انخفض في مجموعة الجرذان المغذاة على عليقة (RP). اللايبوبروتينات ذات الكثافة العالية (HDL) في مصل الدم والحامض الصفراوي في البراز كانت اعلى في الجرذان المغذاة بالعليقة (RP) من الجرذان المغذاة بالعليقتان (C) او (RPM). اشارة هذه النتائج الى ان زيادة افراز الحامض الصفراوي في البراز قد يكون هو المسئول عن التأثير المضاد لارتفاع الكولسترول مع عليقة بروتينات الرز مقارنة مع الكازين.