NUTRITION RESEARCH 36 (2016) 1162–1170

Available online at www.sciencedirect.com

ScienceDirect

www.nrjournal.com

Original Research

Low daily dose of 3 mg monacolin K from RYR reduces the concentration of LDL-C in a randomized, placebo-controlled intervention

Tina Heinz[a], *Jan Philipp Schuchardt*[a], *Katharina Möller*[a], *Peyman Hadji*[b], *Andreas Hahn*[a,*]

[a] *Institute of Food Science and Human Nutrition, Leibniz University Hannover, Germany*
[b] *Department of Gynaecology and Obstetrics, Hospital Nordwest, Frankfurt am Main, Germany*

ARTICLE INFO

Article history:
Received 10 March 2016
Revised 25 June 2016
Accepted 19 July 2016

Keywords:
LDL-cholesterol
Red yeast rice
Monacolin K
Homocysteine
Folic acid

ABSTRACT

Hypercholesterolemia and elevated homocysteine concentrations are associated with cardiovascular risk. Previous studies have demonstrated a cholesterol-lowering effect of red yeast rice (RYR) supplements which contained 5 to 10 mg of monacolin K. We hypothesized that the intake of a low monacolin K dose may likewise reduce low-density lipoprotein–cholesterol (LDL-C) and other plasma lipids. In secondary analyses, we tested the homocysteine lowering effect of folic acid, which was also included in the study preparation. Therefore, we conducted a randomized, double-blind, and placebo-controlled intervention study. One hundred forty-two nonstatin-treated participants with hypercholesterolemia (LDL-C $\geq$ 4.14 $\leq$ 5.69 mmol/L) were randomized to the supplement group with RYR or the placebo group. Participants of the supplement group consumed 3 mg monacolin K and 200 μg folic acid per day. A significant ($P < .001$) reduction of LDL-C (–14.8%), total cholesterol (–11.2%), and homocysteine (–12.5%) was determined in the supplement group after 12 weeks. A total of 51% of the participants treated with RYR achieved the limit of LDL-C <4.14 mmol/L advised and 26% reached the threshold level of homocysteine <10 μmol/L. No significant changes were exhibited within the placebo group. Other parameters remained unchanged and no intolerances or serious adverse events were observed. In conclusion, we demonstrated that a low dose of daily 3 mg monacolin K from RYR reduces the concentration of LDL-C; a risk factor for cardiovascular diseases.

Abbreviations: BMI, body mass index; CVD, cardiovascular diseases; HDL-C, high-density lipoprotein–cholesterol; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL-C, low-density lipoprotein–cholesterol; RYR, red yeast rice; TC, total cholesterol; TG, triacylglycerol.

* *Corresponding author at:* Leibniz University of Hannover, Institute of Food Science and Human Nutrition, Am Kleinen Felde 30, 30167 Hannover, Germany. Tel.: +49 511 762 5969; fax: +49 511 762 5729.
E-mail addresses: heinz@nutrition.uni-hannover.de (T. Heinz), hahn@nutrition.uni-hannnover.de (A. Hahn).

http://dx.doi.org/10.1016/j.nutres.2016.07.005

1. Introduction

Hypercholesterolemia is a well-known risk factor for cardiovascular diseases (CVD) and coronary heart diseases [1,2]. Epidemiological studies have shown a direct correlation between low-density lipoprotein–cholesterol (LDL-C) and the risk of atherosclerosis and myocardial infarction [3]. Reducing total cholesterol (TC) and LDL-C plasma levels are an established and effective strategy in the prevention of coronary events [4,5]. Several meta-analyses indicate that a LDL-C reduction of 1 mmol/L is associated with a 20% to 23% reduction in coronary and vascular disease risks [6,7]. Providing that no other risk factors exist, LDL-C levels of ≥4.14 mmol/L are considered borderline high and should be lowered [3]. Changes in lifestyle, especially dietary modification (increased consumption of unsaturated fatty acids and dietary fibers), and regular physical activities are the primary treatment approach. If other risk factors, such as hypertension, dyslipidemia, or atherosclerotic vascular diseases, exist, a drug treatment with statins or bile acid sequestrant could be necessary. In the case of hypercholesterolemia, balanced diets constitute the main nutritive strategy for lowering blood lipids. Furthermore, enriched food and food supplements (ie, containing phytosterols) are discussed as an additional measure [8-10].

Red yeast rice (RYR) is a traditional food in many Asian countries and a favored ingredient for the preparation of fish, meat, or even rice wine [11]. It has been used to promote digestion as well as blood circulation ever since the Ming Dynasty [12]. Red yeast rice is produced by the fermentation of white rice and the mold fungus *Monascus purpureus*. Red dyestuffs, flavors, and especially components such as monacolins occur according to the fermentation conditions [12]. Monacolins—in particular monacolin K, which has an identical structure to lovastatin [13]—inhibit the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. As a result, the endogenous synthesis of cholesterol is reduced and elevated cholesterol levels decrease. Several intervention studies demonstrated that RYR containing 5 to 10 mg monacolin K lowers elevated LDL-C levels by about 22% to 27% [14-18]. The European Food Safety Authority considers that a daily intake of 10 mg monacolin K from RYR contributes to the maintenance of a normal LDL-C plasma level [19]. However, studies in which participants supplemented RYR products with a low dosage of 3 mg monacolin K plus other cholesterol-lowering agents, such as berberine or policosanols, observed a decrease of the LDL-C level by 20% to 31% [20-24]. However, the supplements used contained additional cholesterol-lowering agents and, because of this combination, it is difficult to determine whether the lipid-lowering effect was achieved mainly by monacolin K or another agent.

In addition to hypercholesterolemia, elevated levels of homocysteine are associated with a high risk of CVD, stroke, and venous thrombosis [25-27]. Decreasing homocysteine levels might be effective in the prevention of CVD [28,29], although several studies failed to show this relationship [30-32]. Homocysteine is a nonessential amino acid, which is converted into methionine or metabolized to cysteine by using the cofactors folic acid and vitamin B_{12} or B_6. Deficits in these vitamins inhibit the degradation and induce an increase of homocysteine (hyperhomocysteinemia). Several studies have shown that elevated homocysteine concentrations could be reduced through a supplementation of folic acid in doses of 0.1 to 10.0 mg [33-35].

Previous intervention studies demonstrated an LDL-C-lowering effect of RYR in doses ranged from 5 to 10 mg/d monacolin K [36,37]. In the present study, the efficacy of a combined preparation with a low dose of monacolin K (3 mg/d) together with a physiological dose of folic acid (200 μg/d) was investigated. We hypothesized a LDL-C lowering-effect in hypercholesterolemic participants after intake of 3 mg/d monacolin K from RYR extract. To test this hypothesis, we conducted a randomized, double-blind, and placebo-controlled intervention study. Our main objective was to determine the plasma level especially of LDL-C at baseline, after 6 and 12 weeks of treatment. Secondary objective was to analyze the homocysteine-lowering effect after intake of 200 μg/d folic acid.

2. Methods and materials

The randomized, placebo-controlled, and double-blind intervention study was planned and conducted according to the set of ethical principles laid down in the Declaration of Helsinki, which was revised for the most recent time in 2013, and to the guidelines of Good Clinical Practice. The study protocol was approved by the Ethical Committee of the Medical Chamber of Lower Saxony (Hannover). The intervention study was registered in the German Clinical Trials Register (identification no. DRKS00006189). The examinations for the nutritional study were carried out from July 2014 to May 2015 at the Institute of Food Science and Human Nutrition, Leibniz University of Hannover, Germany and at the TRIAMEDIS health center, a facility of Hospital Nordwest, Department of Gynecology and Obstetrics, Frankfurt am Main, Germany.

2.1. *Participants*

Male or female participants were recruited by advertisement in daily and weekly newspapers in the greater area of Hannover and Frankfurt am Main. Inclusion criteria were an LDL-C level ≥4.14 mmol/L and ≤5.69 mmol/L and an age between 18 and 70 years. Exclusion criteria were defined as follows: body mass index (BMI), >35 kg/m^2; triacylglycerol (TG) level, ≥4.52 mmol/L; chronic diseases (eg, malignant tumors, manifest CVD, insulin-dependent type 1 and 2 diabetes, severe renal, or liver diseases); chronic gastrointestinal disorders (especially small intestine, pancreas, and liver) and prior gastrointestinal surgical procedures (eg, gastrectomy, coeliac disease, enterocolitis, chronic pancreatitis, cholestasis, short bowel syndrome, chronic inflammatory bowel disease); hormonal disorders (eg, Cushing's syndrome and untreated hyperthyroidism); uncontrolled hypertension; intake of lipid lowering drugs (including statins, fibrates, bile acid binders, nicotinic acid, and Ezetimibe) or supplements (including ω-3 and -6 fatty acids, β-glucans, betaine, ketosan, glucomannan, guar resins, hydropropyl methylcellulose, RYR products, oleic acid, pectins, plant sterols/stanols, and their

esters) during the last 3 months before baseline; change in dosage of the following medications: (oral) corticosteroids, contraceptives, hormone replacement therapy, thiazide diuretics, β-blockers, antidiabetics; myopathy and unexplained muscle pain; pregnancy and breastfeeding; and addiction to alcohol, drugs, and/or medications. All participants gave their written informed consent before the first examination.

2.2. Study design

The study consisted of a screening phase and a 12-week long intervention period with 3 examinations: baseline examination (t_0), intermediate examination after 6 weeks (t_6), and final examination after 12 weeks (t_{12}). Interested participants were informed, filled in an admission questionnaire, and had to show a laboratory result with the current cholesterol levels (not older than 1 year). Potential participants who met the inclusion criteria without showing exclusion criteria were invited to t_0. On the basis of the blood values of t_0 (especially LDL-C and TG), suitable participants were included in the study, 1 day after t_0. All participants received investigational products by post and were informed about the start of intervention (1 week after t_0). Participants ingested either the preparation with RYR or the placebo during the 12-week study. The daily intake of 1 tablet should occur every evening at dinner time with an adequate amount of liquid (eg, water). Anthropometric data (height, body weight, waist and hip circumferences, blood pressure, heart rate) were measured at t_0, t_6, and t_{12}. Participants filled in questionnaires to check the current state of health, medications, physical activity, and the presence of any side effects.

The RYR preparation contained per tablet was 200.0 mg RYR (3.0 mg monacolin K), 2.0 mg coenzyme Q10, 0.5 mg astaxanthin, and 200.0 μg folic acid (Armolipid by Madaus GmbH Köln, Germany—now a part of MEDA Pharma, Bad Homburg, Germany). The RYR preparation was free of citrinin—a nephrotoxic and hepatotoxic mycotoxin which could occur as a by-product due to the fermentation process of RYR [38]. The placebo was similar in appearance and flavor, but without any effective ingredients (Table 1). All participants were randomly assigned in a 1:1 ratio into 2 treatment groups: supplement group (s-group) and placebo group (p-group). Participants were stratified by sex and LDL-C concentration and, therefore, 2 ranges of LDL-C concentrations were determined: low range (4.14-4.89 mmol/L) and high range (4.91-5.69 mmol/L). The investigational products (RYR preparation and placebo) were randomized in a 6-block system by a noninvolved third party. All participants, investigators, physicians, and all the study team members were blinded to treatment allocation throughout the 12-week study. Evaluation of the compliance occurred through counting all tablets returned and comparing the results with the intake recommended. A regular consumption was expected when the quantity of forgotten tablets did not exceed 10%. Participants were classified as compliant if they visited t_0 and 1 further examination, and if they consumed the investigational products regularly. All participants were asked to maintain their usual eating patterns and physical activity during the intervention. Participants had to fill in a 3-day dietary record (1 weekend day and 2 weekdays) at the beginning and end of the study. Nutrition calculations were carried out using PRODI (Nutri-Science GmbH, Freiburg, Germany).

Table 1 – Chemical composition of the RYR preparation and placebo

Ingredients	RYR preparation (mg/tablet)	Placebo (mg/tablet)
Dicalcium phosphate	304.6	–
Dibasic calcium phosphate	–	499.0
Microcrystalline cellulose	253.2	318.3
Monacolin K	3.0	–
Astaxanthin	0.5	–
Magnesium stearate	8.0	8.3
Mono- and diglycerides of fatty acids	8.0	–
Silicon dioxide	4.0	4.1
Coenzym Q10	2.0	–
Pteroyl monoglutamic acid (folic acid)	0.2	–
Red iron oxides	–	8.3
Brown iron oxides	–	2.1

The primary end point of the present intervention study was to observe the reduction of LDL-C in the s-group compared with the p-group over the study duration of 12 weeks. Secondary end points were the determination of the effects on homocysteine and other plasma lipids (TC, high-density lipoprotein–cholesterol [HDL-C], TG), as well as safety parameters and parameters such as fasting glycaemia or high sensitive C-reactive protein.

2.3. Plasma collection and analyses

Venous blood samples were gathered in the morning at t_0, t_6, and t_{12} after an overnight fasting for 12 hours. In total, 50 mL blood was taken from each participant by a qualified physician. The blood samples were stored at +4°C until analyses, which were conducted by an external laboratory (LADR, Hannover, Germany). Plasma lipids (TC, LDL-C, HDL-C, LDL-C/HDL-C ratio, TG) were measured by using an enzymatic colorimetric assay. Homocysteine levels were determined by high-performance liquid chromatography. Creatinine was measured using a kinetic colorimetric assay. Fasting blood glucose was determined by an enzymatic UV method (hexokinase method) and fasting insulin was measured by an immunological in vitro test.

2.4. Statistical analyses

Normal distribution of the whole data set was analyzed by using the Kolmogorov-Smirnov test. The unpaired *t* test was used for normal distributed data to compare the 2 treatment groups and the paired *t* test was used to compare within group differences from t_0. Intragroup and intergroup differences of skewed distributed data were analyzed by using the Mann-Whitney *U* test and Wilcoxon test. The comparison between the treatment groups for categorical variables was performed using the χ^2 test. Expecting a 15% dropout rate, a sample size of 80 participants per group (s- and p-group) would provide 80% power to detect a significant difference between the treatment groups. Differences were considered

significant at a P value ≤.05. Results were presented as mean values ± standard deviation (SD). Moreover, difference between time points was indicated in percentage.

All statistical analyses were carried out in the intention-to-treat population. In case of withdrawal or absence during t_6 or t_{12}, the missing values were substituted by the principle of the last observation carried forward. Statistical Package of Social Sciences 21.0 (SPSS Inc, Chicago, Illionois, USA) was used for the statistical analysis of anthropometric and laboratory chemical data.

3. Results

3.1. Study population

A total of 235 participants were invited to the baseline visit (Fig. 1). One hundred fifty-one participants fulfilled the inclusion and exclusion criteria, were included in the study, and were randomized to the s-group (n = 73) or p-group (n = 78). Nine participants (6.0%) dropped out after t_0 and did not visit the intermediate examination. Thus, 142 men and women were involved in the intention-to-treat analysis. All participants were consistently divided between the 2 treatment groups: s-group (n = 70, 27 males, 43 females) and p-group (n = 72, 26 males, 46 females). In total, more female than male participants were included in the study population; however, the relation between male and female was similar between the 2 groups.

No differences were observed among the treatment groups at t_0 regarding parameters such as sex, age, BMI, hip and waist circumferences, blood pressure, heart rate, and LDL-C levels (Table 2). The analysis of the 3-day dietary records showed no differences between the s-group and p-group at t_0 as well as at the end of the intervention study (data not shown).

3.2. Parameters of lipid metabolism

The mean LDL-C levels of both treatment groups at t_0 were within the range targeted of ≥4.14 mmol/L and ≤5.69 mmol/L (s-group, 4.86 mmol/L; p-group, 4.91 mmol/L), which is considered as borderline-high. The LDL-C levels did not differ between the s-group and p-group at the beginning of the study (P = .433; Fig. 2). The LDL-C concentration decreased significantly (–14.8%; P < .001) in the s-group after 12 weeks (primary end point). The LDL-C concentration of the s-group was significantly (P < .001) reduced by 14.9% after 6 weeks and reached a level of 4.13 mmol/L at t_6, which was constant until the end of the study (Fig. 2). A total of 53% of the participants in the s-group reached the LDL-C threshold level of <4.14 mmol/L [3] after 6 weeks of intervention (t_6) and 51% achieved this level after 12 weeks of treatment (t_{12}). The LDL-C levels in the p-group were reduced by 2.7% at t_{12}; however, the change was not significant. In comparison with the p-group, the LDL-C concentrations of the s-group were significantly (P < .001) different at t_6 and t_{12}.

The levels of further plasma lipids, such as TC, HDL-C, and LDL-C/HDL-C ratio, did not differ between the s-group and p-group at the beginning of study (Table 3). The TG level of the s-group was 7.0% higher than in the p-group at t_0, whereby this difference was not significant. The lowered LDL-C

Table 2 – Anthropometric and biochemical characteristics of the study participants at baseline (t_0)

	Total group	S-group	P-group	P value
Number of participants	142	70	72	
Sex distribution (m/f)	53:89	27:43	26:46	.762#
Age (y)	57.3 ± 7.0	57.5 ± 7.2	57.0 ± 6.8	.645†
Weight (kg)	77.5 ± 15.4	78.4 ± 16.2	76.6 ± 14.7	.570†
Height (m)	1.71 ± 0.10	1.70 ± 0.09	1.72 ± 0.10	.296‡
BMI (kg/m^2)	26.4 ± 4.1	26.9 ± 4.2	25.9 ± 3.9	.163†
WC (cm)	90.9 ± 12.4	92.3 ± 12.7	89.5 ± 12.1	.247†
HipC (cm)	103.0 ± 9.3	103.6 ± 9.4	102.4 ± 9.1	.469†
SBP (mm Hg)	130.7 ± 17.2	131.2 ± 17.2	130.3 ± 17.3	.643‡
DBP (mm Hg)	79.5 ± 10.3	78.7 ± 8.4	80.3 ± 11.9	.443‡
Heart rate (bpm)	68.0 ± 8.1	67.9 ± 9.0	68.1 ± 7.1	.960‡
LDL-C (mmol/L)	4.88 ± 0.43	4.86 ± 0.41	4.91 ± 0.44	.433‡

Values are shown as means ± SD; s-group, supplement-group; p-group, placebo-group; m, male participants; f, female participants; BMI, body mass index; WC, waist circumference; HipC, hip circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, LDL-cholesterol; # χ^2 test; † t test; ‡ Mann-Whitney U test.

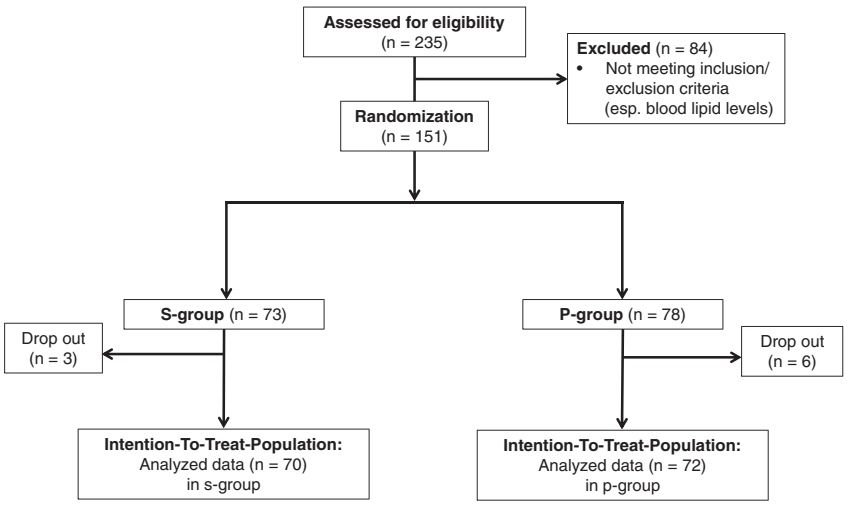

Fig. 1 – Flow diagram of the study population. n indicates number of participants.

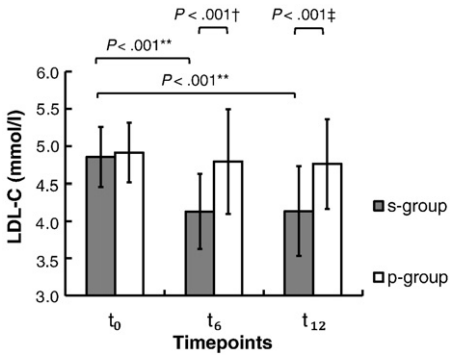

Fig. 2 – Low-density lipoprotein-cholesterol concentration at baseline (t_0), after 6 (t_6) and 12 (t_{12}) weeks of intervention. Values are shown as means ± SD; s-group, supplement-group; p-group, placebo-group; LDL-C, LDL-cholesterol; † t test for unpaired data; ‡ Mann-Whitney *U* test; ** Wilcoxon test.

levels were associated with the improvement of TC concentration and LDL-C/HDL-C ratio in the s-group. The TC levels in the s-group decreased significantly by 11.2% and the LDL-C/HDL-C ratio was lowered by –14.1% (Table 3). These differences occurred mainly in the first 6 weeks of intervention and both parameters at least reached a constant level during the period between t_6 and t_{12}. No significant differences in TC (–1.0%; *P* = .515) and LDL-C/HDL-C ratio (–2.2%; *P* = .116) were observed within the p-group during the whole intervention period. Furthermore, HDL-C levels remained unchanged in the s-group and p-group throughout the entire duration of the study (Table 3). The level of TG was reduced by 5.0% in the s-group from t_0 to t_{12} (*P* = .001). However, differences between the TG concentrations of the s-group and p-group were not significant at any time point.

Participants of the s-group were separated into a subgroup with slightly elevated LDL-C levels (4.14-4.89 mmol/L) and compared with a subgroup with high LDL-C levels (4.91-5.96 mmol/L) at baseline t_0 (Table 4). The LDL-C-lowering was stronger in s-group participants with higher LDL-C levels (17.1%) compared with slightly elevated LDL-C levels at t_0, where the relative LDL-C reduction was 12.8%.

3.3. *Homocysteine*

The levels of homocysteine were similarly elevated in both treatment groups at t_0 (*P* = .797) and were above the normal plasma homocysteine level of <10 μmol/L (Table 5). The homocysteine concentrations of the s-group were significantly reduced by 12.5% and reached a mean of 10.2 μmol/L at t_{12}. The homocysteine level at the beginning of the study (t_0) was >10 μmol/L in 76% of the participants in the s-group, whereas 50% of the participants reached the threshold level of <10 μmol/L at t_{12}. Thus, the homocysteine concentration of 26% of the s-group participants was lowered and reached the reference range. No changes of the homocysteine values were observed within the p-group during the whole intervention period.

3.4. *Safety parameters, adverse events, and compliance*

All safety parameters (creatinine, creatine kinase, uric acid, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase) analyzed as well as fasting glucose, fasting insulin, HbA1c, and high sensitive C-reactive protein showed neither undesirable changes in response to RYR treatment nor significant differences between the 2 treatment groups at any time point. All values were within the reference ranges throughout the whole study duration.

No serious adverse events were observed during the intervention period of 12 weeks. Flatulence was the most common adverse event in the s-group and p-group (1% of the whole study population), which occurred with similar frequency in both treatment groups. Diarrhea, obstipation, and abdominal fullness occurred seldom and in the s-group as frequently as in the p-group. Belching was more common in the s-group at t_{12} (with a difference of 6%).

Three of the 9 dropouts received the preparation with RYR and 6, the placebo. Participants who took the RYR preparation dropped

Table 3 – Serum lipid concentrations at baseline (t_0), after 6 (t_6) and 12 (t_{12}) weeks of intervention

Lipid parameter	Treatment groups	t_0	t_6	t_{12}	t_{12}-t_0	
					Difference	% Difference
TC (mmol/L)	S-group (n = 70)	7.00 ± 0.65	6.25 ± 0.73**	6.20 ± 0.74**	–0.80 ± 0.66	–11.2 ± 9.2
	P-group (n = 72)	6.97 ± 0.67	6.88 ± 0.88	6.88 ± 0.90	–0.09 ± 0.78	–1.0 ± 11.2
	P value (s-group vs p-group)	.833‡	<.001‡	<.001†		
HDL-C (mmol/L)	S-group (n = 70)	1.64 ± 0.42	1.64 ± 0.42	1.65 ± 0.44	0.01 ± 0.20	0.7 ± 12.3
	P-group (n = 72)	1.59 ± 0.36	1.57 ± 0.41	1.60 ± 0.40	0.00 ± 0.18	0.2 ± 11.3
	P value (s-group vs p-group)	.583‡	.394‡	.438†		
LDL-C/HDL-C ratio	S-group (n = 70)	3.1 ± 0.8	2.7 ± 0.8**	2.7 ± 0.9**	–0.4 ± 0.4	–14.1 ± 13.5
	P-group (n = 72)	3.3 ± 0.8	3.2 ± 0.9	3.2 ± 0.9	–0.1 ± 0.4	–2.2 ± 12.6
	P value (s-group vs p-group)	.409‡	<.001‡	.001‡		
TG (mmol/L)	S-group (n = 70)	1.59 ± 0.78	1.43 ± 0.67*	1.49 ± 0.93*	–0.10 ± 0.69	–5.0 ± 40.6
	P-group (n = 72)	1.47 ± 0.63	1.55 ± 0.67	1.42 ± 0.66	–0.05 ± 0.49	–0.4 ± 28.4
	P value (s-group vs p-group)	.487‡	.150‡	.700‡		

Values are shown as means ± SD; s-group, supplement-group; p-group, placebo-group; n, number of participants; TC, total cholesterol; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; TG, triacylglycerides; %, percentage of difference; † *t* test; ‡ Mann-Whitney *U* test; * *P* < .01 and ** *P* < .001 analyzed by Wilcoxon test.

Table 4 – Changes in lower and higher LDL-C subgroups of the supplement group at baseline (t_0) and after 12 (t_{12}) weeks of intervention

s-group	t_0 (mmol/L)	t_{12} (mmol/L)	Difference t_{12}-t_0	
			(mmol/L)	%
Lower LDL-C group: 4.14-4.89 mmol/L (n = 38)	4.54 ± 0.22	3.95 ± 0.53**	–0.58 ± 0.50	–12.8 ± 11.2
Higher LDL-C group: 4.91-5.69 mmol/L (n = 32)	5.24 ± 0.21	4.34 ± 0.54**	–0.89 ± 0.50	–17.1 ± 9.5

Values are shown as means ± SD; s-group, supplement-group; n, number of participants; LDL-C, LDL-cholesterol; %, percentage of difference; ** $P < .001$ analyzed by Wilcoxon test.

out after a participation of 2 or 3 days and mentioned diarrhea and restless sleep as reasons. Participants of the p-group gave the following reasons for interrupting the intervention: restless sleep, stomach problems, joint and muscle pain, exhaustion, fatigue, and operation/influenza (independent of the study).

A total of 90.8% (n = 129) of the participants were compliant and 9.2% (n = 13) were not compliant.

4. Discussion

Previous studies with RYR and the daily monacolin K dosages between 5 and 10 mg proved an LDL-C-lowering effect in the range of 20% to 30% [37,39-43]. In the present study, we show that the RYR extract tested in a daily dose of only 3 mg monacolin K led to a significant cholesterol-lowering effect. The LDL-C level was reduced by about 15% in the s-group compared with the p-group during the whole study duration. The mean LDL-C level in the s-group dropped under the National Cholesterol Education Program limit advised of <4.14 mmol/L [3] after 6 and 12 weeks (4.13 mmol/L at each time point) of RYR treatment. More than half of the participants supplemented with RYR reached that National Cholesterol Education Program treatment goal, most of them after a treatment of 6 weeks. Together, these data show that borderline high LDL-C levels from nonstatin-treated participants can be effectively lowered despite the low dosage of 3 mg monacolin K in the RYR extract used.

Most studies with a total intervention time of 8 or 12 weeks showed a considerable LDL-C-lowering effect of RYR supplementation after 4 or 8 weeks of treatment [14,15,17]. A constant level of LDL-C is probably reached rapidly after a few weeks and remains consistent until the end of the study. The inhibiting effect on HMG-CoA reductase might occur due to a saturated mechanism. Monacolin K inhibits the activity of HMG-CoA reductase and the endogenous cholesterol synthesis is impaired [44]. Because less endogenous cholesterol is produced, the cholesterol level decreases slowly and a constant level is reached after a few weeks and remains consistent. Further studies including tight measurement time points are required to investigate the time point of the maximal reduction and LDL-C steady state with a certain monacolin K dose from RYR. It should be noted that the LDL-C-reducing effect of monacolin K from RYR could be dependent on the dose administered and on the basal LDL-C levels. Heber et al (1999), for example, demonstrated that a daily intake of 7.5 mg monacolin K lowered the LDL-C levels by 22% after a treatment of 12 weeks [14]. In our study, we proved a LDL-C reduction of about 15% in response to a supplementation of 3 mg monacolin K within 12 weeks. The basal LDL-C concentrations of both intervention studies were ≥4.14 mmol/L. Moreover, we observed in the present study a greater LDL-C reduction in participants with higher basal LDL-C concentrations (4.91-5.69 mmol/L) compared with participants with lower LDL-C levels (4.14-4.89 mmol/L) in response to RYR intake. Further studies including different doses of monacolin K and a separation of the participants in subgroups with different basal LDL-C levels are required to investigate the cholesterol-lowering effect.

Equally, the opposite question arises whether the LDL-C reduction maintains over a long-term period. A trial with a daily intake of 10.0 to 12.8 mg monacolin K suggested a constant LDL-C reduction of 20% over a period of 4.5 years [16]. However, further long-term studies are necessary to confirm these results and to evaluate the long-term tolerability of RYR supplements with low and high monacolin K doses from RYR extracts. No intolerances or serious adverse events were observed during the intervention with a low dosage of monacolin K (3 mg/d) in the present study.

It is difficult to compare intervention studies with RYR extracts, because most studies only indicate the RYR dosage without giving information on the daily monacolin K dose. However, monacolin K is the key agent in RYR extract to lower cholesterol levels by inhibiting the activity of HMG-CoA reductase, resulting in an inhibition of the endogenous cholesterol synthesis [44]. Thus, the monacolin K dose is required to interpret the efficacy of RYR extracts among studies. Furthermore, the concentration of monacolin K can vary in supplements with a similar amount of RYR. Heber et al (2001) investigated 9 Chinese supplements containing RYR and found that the monacolin K content ranged from 0.15 to 3.37 mg/capsule [38]. It is recommended that further studies with RYR extract indicate the monacolin K dose clearly.

Because of the fermentation process, RYR contains additional bioactive compounds. In addition to monacolin K, these

Table 5 – Homocysteine at baseline (t_0) and after 12 (t_{12}) weeks of intervention

Treatment groups	t_0 (μmol/L)	t_{12} (μmol/L)	Difference t_{12}-t_0	
			(μmol/L)	%
s-group (n = 70)	12.0 ± 4.2	10.2 ± 2.3**	–1.9 ± 3.2	–12.5 ± 14.6
p-group (n = 72)	11.7 ± 2.6	11.6 ± 3.3	–0.1 ± 2.4	–0.4 ± 20.6
P value (s- vs p-group)	.797‡	.002‡		

Values are shown as means ± SD; s-group, supplement-group; p-group, placebo-group; n, number of participants; %, percentage of difference; ‡ Mann-Whitney *U* test; ** $P < .001$ analyzed by Wilcoxon test.

include 13 other monacolins as well as their hydroxy acid forms, fiber, unsaturated fatty acids, plant sterols, isoflavones, and isoflavone glycosides [11,12,45]. Compounds such as fiber and plant sterols contribute to the cholesterol-lowering effect [46,47] and may act synergistically in the RYR [11,41,48]. Monacolin K is considered as the most effective among all compounds in the reduction of cholesterol levels.

An effect of coenzyme Q10 on the cholesterol metabolism was observed in the murine model [49,50]. However, several human intervention studies demonstrated no beneficial effects on LDL-C levels or other lipid parameters after supplementing doses of 200 mg/d coenzyme Q10 over 3 to 6 months [51-53]. In our study, the coenzyme Q10 dose was 100-fold lower (2.0 mg/d). Hence, a cholesterol-lowering effect of coenzyme Q10 is unlikely.

The homocysteine-lowering effect of folic acid has been shown in various studies in concentrations of –3.0 to –4.4 μmol/L; however, it ranged from 2.5 to 10.0 mg folic acid per day in pharmacological doses [33,54,55]. In the present study, we observed a significant homocysteine-lowering effect (–12.5%) after an intervention of 12 weeks with a physiological folic acid concentration (200 μg a day). A total of 26% of the participants from the s-group reached the reference value of <10 μmol/L recommended, whereas no changes in the homocysteine concentration were observed in participants of the p-group. Comparable intervention trials supplementing folic acid in similar concentrations (200-250 μg a day) to participants with similar basal homocysteine levels showed a homocysteine reduction of about 10% to 12% [35,56,57]. Thus the effect size in these studies is comparable with our findings.

The study has a number of potential limitations. Potential antioxidant effects of astaxanthin and coenzyme Q10 (eg, on oxidized LDL-C), which were also included in the study preparation, were not evaluated. However, because of the low dosage of coenzyme Q10 and astaxanthin, antioxidant effects are rather not to be expected. Likewise, the endogenous levels of folic acid were not analyzed, which would have been useful to elevate the supply status of this B vitamin. The strengths of our study rely on the high sample size, the relatively long duration and the straightforward design. Similarly, our study yielded data on the safety and tolerability of the RYR preparation used.

In conclusion, our study demonstrated that a relatively low content of RYR (3 mg monacolin K per day) can reduce elevated concentrations of LDL-C in nonstatin-treated participants significantly. Thus, these results confirm our hypothesis. Furthermore, a physiological dose of folic acid (200 μg per day) can lower elevated homocysteine levels significantly. Hence, the study preparation used is effective in lowering cholesterol and homocysteine and may be practically integrated in a primary CVD and coronary heart diseases prevention strategy.

Acknowledgment

First of all, we thank the participants who contributed their time to this project. The study was partly supported by Madaus GmbH, Köln, Germany (now a part of MEDA Pharma, Bad Homburg, Germany). The authors are solely responsible for the design and conduct of the study, collection, management, analysis, and interpretation of the data, as well as preparation of the manuscript. All authors had full access to the data and take responsibility for its integrity. All authors have read and agreed with the manuscript as written. There was no conflict of interest for all authors.

REFERENCES

[1] Sharma RK, Singh VN, Reddy H. Thinking beyond low-density lipoprotein cholesterol: strategies to further reduce cardiovascular risk. Vasc Health Risk Manag 2009;5:793–9.
[2] Farzadfar F, Finucane MM, Danaei G, et al. National, regional, and global trends in serum total cholesterol since 1980: systematic analysis of health examination surveys and epidemiological studies with 321 country-years and 3·0 million participants. Lancet 2011; 9765:578–86.
[3] NCEP III. Third report on the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III) final report. Circulation 2002;3143-421.
[4] Wilson P, Castelli W, Kannel W. Coronary risk prediction in adults (the Framingham Heart study). Am J Cardiol 1987;14: 91G–94.
[5] Cholesterol Treatment Trialists' (CTT) Collaborators. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90 056 participants in 14 randomised trials of statins. Lancet 2005; 9493:1267–78.
[6] Law MR, Wald NJ, Rudnicka AR. Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis. BMJ 2003;326:1423.
[7] Robinson JG, Smith B, Maheshwari N, et al. Pleiotropic effects of statins: benefit beyond cholesterol reduction? A meta-regression analysis. J Am Coll Cardiol 2005;10:1855–62.
[8] Anderson JW, Allgood LD, Lawrence A, et al. Cholesterol-lowering effects of psyllium intake adjunctive to diet therapy in men and women with hypercholesterolemia: meta-analysis of 8 controlled trials. Am J Clin Nutr 2000; 2:472–9.
[9] Rajaram S, Burke K, Connell B, et al. A monounsaturated fatty acid–rich pecan-enriched diet favorably alters the serum lipid profile of healthy men and women. J Nutr 2001; 9:2275–9.
[10] Devaraj S, Jialal I, Vega-López S. Plant sterol-fortified orange juice effectively lowers cholesterol levels in mildly hypercholesterolemic healthy individuals. Arterioscler Thromb Vasc Biol 2004;3:e25–8.
[11] Wang J, Lu Z, Chi J, et al. Multicenter clinical trial of the serum lipid-lowering effects of a monascus purpureus (red yeast) rice preparation from traditional chinese medicine. Curr Ther Res 1997;12:964–78.
[12] Ma J, Li Y, Ye Q, et al. Constituents of red yeast Rice, a traditional Chinese food and medicine. J Agric Food Chem 2000;11:5220–5.
[13] Hong MY, Seeram NP, Zhang Y, et al. Anticancer effects of Chinese red yeast rice versus monacolin K alone on colon cancer cells. J Nutr Biochem 2008;7:448–58.
[14] Heber D, Yip I, Ashley JM, et al. Cholesterol-lowering effects of a proprietary Chinese red-yeast rice dietary supplement. Am J Clin Nutr 1999;2:231–6.
[15] Lin C, Li T, Lai M. Efficacy and safety of Monascus Purpureus went rice in subjects with hyperlipidemia. Eur J Endocrinol 2005;5:679–86.
[16] Lu Z, Kou W, Du B, et al. Effect of Xuezhikang, an extract from red yeast Chinese rice, on coronary events in a Chinese population with previous myocardial infarction. Am J Cardiol 2008;12:1689–93.

[17] Huang C, Li T, Lin C, et al. Efficacy of Monascus Purpureus went rice on lowering lipid ratios in hypercholesterolemic patients. Eur J Cardiovasc Prev Rehabil 2007; 3:438–40.
[18] Cicero AFG, Derosa G, Parini A, et al. Red yeast rice improves lipid pattern, high-sensitivity C-reactive protein, and vascular remodeling parameters in moderately hypercholesterolemic Italian subjects. Nutr Res 2013;8:622–8.
[19] EFSA Panel. EFSA panel on dietetic products, nutrition and allergies (NDA) scientific opinion on the substantiation of health claims related to monacolin K from red yeast rice and maintenance of normal blood LDL-cholesterol concentrations (ID 1648, 1700) pursuant to article 13(1) of regulation (EC) no 1924/2006. EFSA J 2011;7 [2304,16 pp.].
[20] Cicero A, Benvenuti C. Efficacy of a red yeast rice based nutraceutical in large subgroups of hypercholesterolemic subjects in every day clinical practice. Med J Nutrition Metab 2010;239-46.
[21] Kasliwal RR, Bansal M, Gupta R, et al. ESSENS dyslipidemia: a placebo-controlled, randomized study of a nutritional supplement containing red yeast rice in subjects with newly diagnosed dyslipidemia. Nutrition 2016; 7–8:767–76.
[22] Ruscica M, Gomaraschi M, Mombelli G, et al. Nutraceutical approach to moderate cardiometabolic risk: results of a randomized, double-blind and crossover study with Armolipid plus. J Clin Lipidol 2014;1:61–8.
[23] Pirro M, Lupattelli G, Del Giorno R, et al. Nutraceutical combination (red yeast rice, berberine and policosanols) improves aortic stiffness in low-moderate risk hypercholesterolemic patients. PharmaNutrition 2013;2:73–7.
[24] Affuso F, Ruvolo A, Micillo F, et al. Effects of a nutraceutical combination (berberine, red yeast rice and policosanols) on lipid levels and endothelial function randomized, double-blind, placebo-controlled study. Nutr Metab Cardiovasc Dis 2010;9:656–61.
[25] Graham I, Daly L, Refsum H, et al. Plasma homocysteine as a risk factor for vascular disease. The European concerted action project. JAMA 1997:1775–81.
[26] Stubbs PJ, Al-Obaidi MK, Conroy RM, et al. Effect of plasma homocysteine concentration on early and late events in patients with acute coronary syndromes. Circulation 2000;6:605–10.
[27] Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. BMJ 2002;325:1202.
[28] Boushey C, Beresford S, Omenn G, et al. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA 1995;13:1049–57.
[29] Qin X, Huo Y, Xie D, et al. Homocysteine-lowering therapy with folic acid is effective in cardiovascular disease prevention in patients with kidney disease: a meta-analysis of randomized controlled trials. Clin Nutr 2013;5:722–7.
[30] Bønaa KH, Njølstad I, Ueland PM, et al. Homocysteine lowering and cardiovascular events after acute myocardial infarction. N Engl J Med 2006;15:1578–88.
[31] Ebbing M, Bleie Ø, Ueland P, et al. Mortality and cardiovascular events in patients treated with homocysteine-lowering B vitamins after coronary angiography: a randomized controlled trial. JAMA 2008;7:795–804.
[32] Study of the effectiveness of additional reductions in cholesterol and homocysteine (SEARCH) Collaborative Group. Effects of homocysteine-lowering with folic acid plus vitamin B12 vs placebo on mortality and major morbidity in myocardial infarction survivors: a randomized trial. JAMA 2010;24:2486–94.
[33] Landgren F, Israelsson B, Lindgren A, et al. Plasma homocysteine-lowering effect of folic acid. J Intern Med 1995; 4:381–8.
[34] den Heijer M, Brouwer I, Bos G, et al. Vitamin supplementation reduces blood homocysteine levels: a controlled trial in patients with venous thrombosis and healthy volunteers. Arterioscler Thromb Vasc Biol 1998;356-61.
[35] Venn BJ, Mann JI, Williams SM, et al. Assessment of three levels of folic acid on serum folate and plasma homocysteine: a randomised placebo-controlled double-blind dietary intervention trial. Eur J Clin Nutr 2002;8:748–54.
[36] Becker DJ. Red yeast Rice for dyslipidemia in statin-intolerant patients. Ann Intern Med 2009;12:830–9.
[37] Bogsrud M, Ose L, Langslet G, et al. HypoCol (red yeast rice) lowers plasma cholesterol - a randomized placebo controlled study. Scand Cardiovasc J 2010;4:197–200.
[38] Heber D, Lembertas A, Lu Q, et al. An analysis of nine proprietary Chinese red yeast Rice dietary supplements: implications of variability in chemical profile and contents. J Altern Complement Med 2001;2:133–9.
[39] Zhao SP, Liu L, Cheng YC, et al. Xuezhikang, an extract of cholestin, protects endothelial function through antiinflammatory and lipid-lowering mechanisms in patients with coronary heart disease. Circulation 2004;8: 915–20.
[40] Cicero AFG, Brancaleoni M, Laghi L, et al. Antihyperlipidaemic effect of a Monascus Purpureus brand dietary supplement on a large sample of subjects at low risk for cardiovascular disease: a pilot study. Complement Ther Med 2005;4:273–8.
[41] Halbert SC, French B, Gordon RY, et al. Tolerability of red yeast rice (2,400 mg twice daily) versus pravastatin (20 mg twice daily) in patients with previous statin intolerance. Am J Cardiol 2010;2:198–204.
[42] Verhoeven V, Lopez HM, Remmen R, et al. Red yeast rice lowers cholesterol in physicians - a double blind, placebo controlled randomized trial. BMC Complement Altern Med 2013;13:178.
[43] Moriarty PM, Roth EM, Karns A, et al. Effects of Xuezhikang in patients with dyslipidemia: a multicenter, randomized, placebo-controlled study. J Clin Lipidol 2014;6:568–75.
[44] Endo A. Monacolin K, a new hypo-cholesterolemic agent produced by a monascus species. J Antibiot (Tokyo) 1979;8: 852–4.
[45] Li Y, Zhang F, Wang Z, et al. Identification and chemical profiling of monacolins in red yeast rice using high-performance liquid chromatography with photodiode array detector and mass spectrometry. J Pharm Biomed Anal 2004; 5:1101–12.
[46] Brown L, Rosner B, Willett WW, et al. Cholesterol-lowering effects of dietary fiber: a meta-analysis. Am J Clin Nutr 1999; 1:30–42.
[47] Vanstone CA, Raeini-Sarjaz M, Parsons WE, et al. Unesterified plant sterols and stanols lower LDL-cholesterolconcentrations equivalently in hypercholesterolemic persons. Am J Clin Nutr 2002;6:1272–8.
[48] Gordon RY, Becker DJ. The role of red yeast rice for the physician. Curr Atheroscler Rep 2011;1:73–80.
[49] Ahmadvand H, Ghasemi-Dehnoo M. Antiatherogenic, hepatoprotective, and hypolipidemic effects of coenzyme Q10 in alloxan-induced type 1 diabetic rats. ARYA Atheroscler 2014;10:192–8.
[50] Schmelzer C, Okun JG, Haas D, et al. The reduced form of coenzyme Q10 mediates distinct effects on cholesterol metabolism at the transcriptional and metabolite level in SAMP1 mice. IUBMB Life 2010;11:812–8.
[51] Mohseni M, Vafa M, Zarrati M, et al. Beneficial effects of coenzyme Q10 supplementation on lipid profile and Intereukin-6 and intercellular adhesion molecule-1 reduction, preliminary results of a double-blind trial in acute myocardial infarction. Int J Prev Med 2015; 6:73.

[52] Hamilton SJ, Chew GT, Watts GF. Coenzyme Q10 improves endothelial dysfunction in statin-treated type 2 diabetic patients. Diabetes Care 2009;5:810–2.
[53] Chew GT, Watts GF, Davis TME, et al. Hemodynamic effects of fenofibrate and coenzyme Q10 in type 2 diabetic subjects with left ventricular diastolic dysfunction. Diabetes Care 2008;8:1502–9.
[54] Homocysteine Lowering Trialists' Collaboration. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. BMJ 1998;7135:894–8.
[55] Moat S, Madhavan A, Taylor S, et al. High- but not low-dose folic acid improves endothelial function in coronary artery disease. Eur J Clin Invest 2006;12:850–9.
[56] Brouwer I, van Dusseldorp M, Thomas C, et al. Low-dose folic acid supplementation decreases plasma homocysteine concentrations: a randomized trial. Am J Clin Nutr 1999;69:99–104.
[57] Brouwer I, van Rooij I, van Dusseldorp M, et al. Homocysteine-lowering effect of 500 μg folic acid every other day versus 250 μg/day. Ann Nutr Metab 2000;44:194–7.