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Applied nutritional investigation

## ESSENS dyslipidemia: A placebo-controlled, randomized study of a nutritional supplement containing red yeast rice in subjects with newly diagnosed dyslipidemia



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### ABSTRACT

**Objective:** Evidence suggests prolonged exposure to lower levels of low-density lipoprotein cholesterol (LDL-C), starting at a younger age, substantially lowers cardiovascular (CV) risk. Accordingly, the CV pandemic affecting younger population in low- to low-middle-income countries, where statin usage is poor even in secondary prevention, may benefit from lipid-lowering nutritional products, as nutritional intervention is generally preferred in these cultures. However, the safety and efficacy of such preparations have not been systematically tested.

**Methods:** In this multicenter, double-blind study, 191 statin-free subjects with newly-diagnosed hyperlipidemia (LDL-C >120 mg/dL, 3.11 mmol/L) and no evidence of CV disease were randomized to one capsule of a proprietary bioactive phytonutrient formulation containing red yeast rice, grape-seed, niacinamide, and folic acid (RYR-NS) or matched placebo twice daily, along with lifestyle modification, for 12 wk.

**Results:** Mean baseline LDL-C levels were  $148.5 \pm 24.0$  mg/dL ( $3.85 \pm 0.62$  mmol/L) and  $148.6 \pm 21.9$  mg/dL ( $3.85 \pm 0.57$  mmol/L) in the RYR-NS and placebo groups respectively. Compared with placebo, RYR-NS resulted in a significant reduction in LDL-C ( $-29.4\%$  versus  $-3.5\%$ ,  $P < 0.0001$ ) and non-high-density lipoprotein cholesterol (non-HDL-C;  $-29.8\%$  versus  $-10.3\%$ ,  $P < 0.0001$ ) at 12 wk. With RYR-NS, 43.4% individuals attained desirable LDL-C levels and 55.4% desirable non-HDL-C levels by week 12, compared to only 0% and 1.1%, respectively, at baseline. No safety issues were observed.

**Conclusion:** This study demonstrates the efficacy and safety of RYR-NS in lowering LDL-C and non-HDL-C after 12 wk, with magnitude of LDL-C reduction being comparable to that seen with moderate-intensity statin therapy. Further long-term studies are required to determine the impact of RYR-NS on treatment adherence and clinical outcomes.

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## Introduction

The cardiovascular (CV) disease (CVD) pandemic has led to an unprecedented increase in morbidity and mortality, especially in low- and lower-middle-income countries [1,2]. In these populations, a higher prevalence of CVD risk factors at a younger age coupled with genetic predisposition are the predominant reasons for the rapidly increasing CVD burden [3]. Of the major CVD risk factors, dyslipidemia remains the single-most important factor because of its high prevalence and direct pathogenic association with atherosclerosis [4].

Large-scale epidemiologic and clinical studies have shown that lowering of low-density lipoprotein cholesterol (LDL-C) levels through pharmacologic and/or non-pharmacologic means is associated with significant CV benefits, with each mmol/L reduction in LDL-C levels leading to an approximately 30% reduction in the risk of adverse CV events [5–10]. These beneficial effects of LDL-C reduction are seen irrespective of the

clinical setting and baseline LDL-C levels. Additionally, increasing evidence also suggests that the duration of exposure to low LDL-C levels may be as important as the magnitude of LDL-C lowering [11–16]. A large meta-analysis by Ference et al. has demonstrated that prolonged exposure to lower LDL-C levels beginning at a young age is associated with a substantially greater reduction in the risk of CVD than the current practice of lowering LDL-C levels later in life [13]. At the same time, convincing data is also available to demonstrate the safety of very low LDL-C levels [17–19]. These evidences together suggest that the effectiveness of LDL-C lowering for primary prevention of CVD can be potentially and substantially improved by initiating LDL-C lowering therapies much earlier in life than what is currently practiced. These observations are particularly relevant for developing countries where CVD epidemic is growing exponentially and the healthcare infrastructure is grossly unequipped to manage the resultant morbidity and mortality burden.

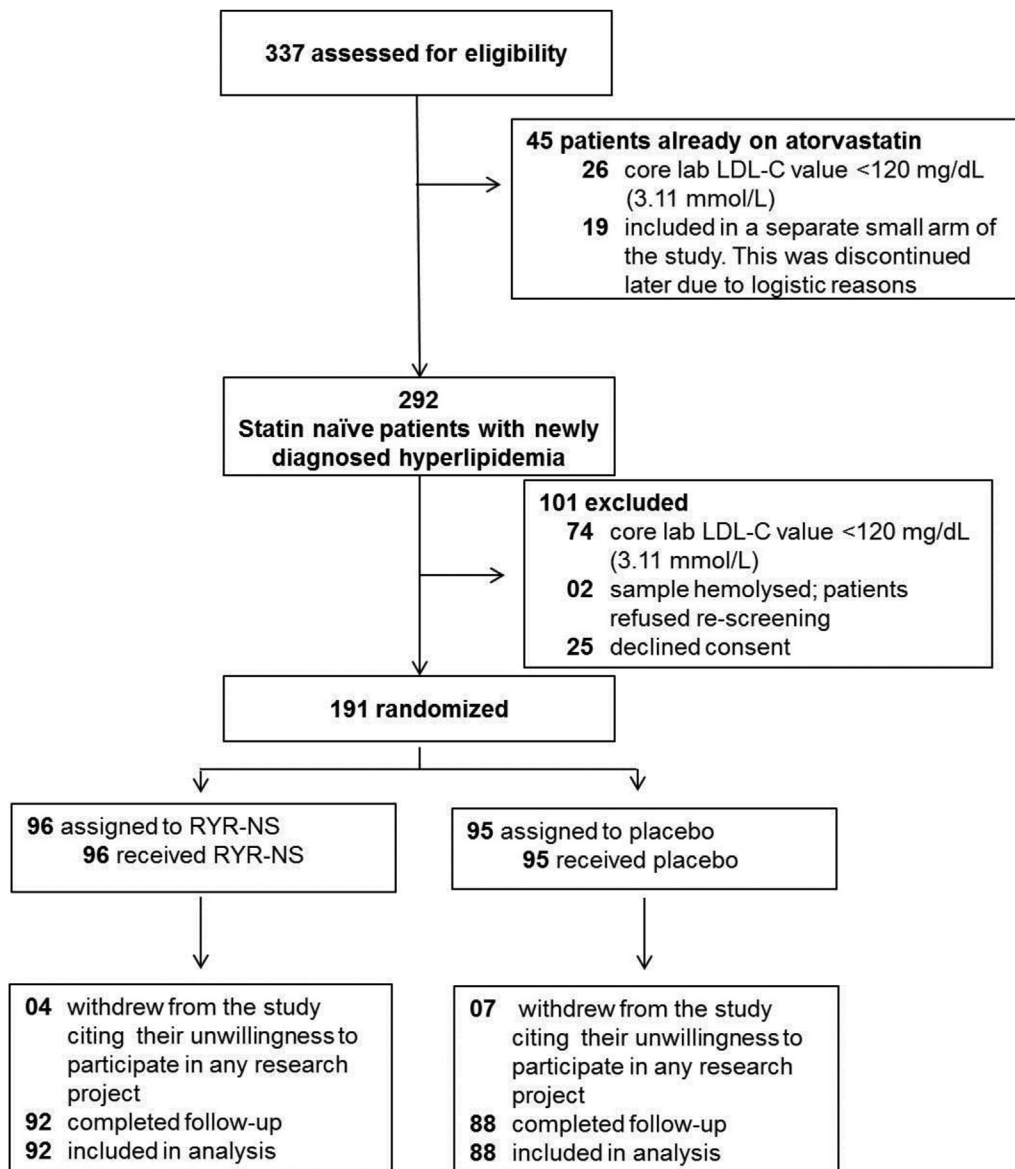


Fig. 1. CONSORT diagram depicting flow of the patients through the trial. LDL-C, low density lipoprotein cholesterol.

However, despite the growing evidence emphasizing the need for early and effective LDL-C lowering, the best approach to achieve this is not clearly known. Although statins are the most effective lipid-lowering agents with unequivocal CV risk reduction ability, their long-term usage generally remains poor, with less than 5% of patients with a previous CV event continuing on statin therapy beyond 5 y in low- and low-middle-income countries [20]. Interestingly, the use of statins (and other proven therapies) has been shown to be much lower in younger individuals compared to the older ones [20], suggesting that the concern of adverse effects and an inherent psychological barrier against being on a “drug for life” are among the major reasons for such a low uptake of statins. These apprehensions are even greater in primary prevention settings where long-term therapy is required and the potential benefits of the treatment are not immediately apparent. In these circumstances, a food-derived formulation that could safely and effectively lower LDL-C levels could potentially lead to significant public health benefits by being perceived as a “softer” therapeutic option and therefore being culturally more acceptable. However, while there are many dietary supplements that claim to exhibit lipid-lowering efficacy, none are backed with the rigor of randomized, double-blind clinical trial evidence. Therefore, this study was sought to evaluate lipid-lowering efficacy and safety of RYR-NS, a proprietary bioactive phytonutrient formulation containing red yeast rice (RYR) powder, grapeseed powder, niacin, folic acid, and black pepper seed powder (Appendix A). RYR has a mechanism of action similar to that of statins [21–24], whereas niacin and grapeseed powder are known to improve lipid profile through different mechanisms [25–27]. This formulation is currently available under the brand name PreLipid (Prevention Meds, Inc, Lebanon, NJ, USA). This study was performed as part of Evaluation Series on Safety & Efficacy of Nutritional Supplements (ESSENS).

## Methods

### Study design

This was a multicenter, randomized, double-blinded, placebo controlled study (Fig. 1) conducted at nine clinical sites across India (Appendix B). At all nine sites, the study was approved by the respective institutional review boards and

the local independent ethics committees. Written informed consent was obtained from each participant before enrolment in the study. The first patient was enrolled on 10 March 2014 and the last patient's final visit took place on November 2, 2014. The study was registered with Clinical Trials.Gov (#NCT02187757).

### Participants

Treatment-free patients ages 18 to 65 y with newly diagnosed hyperlipidemia (LDL-C >120 mg/dL or 3.11 mmol/L) were eligible to participate in the study. We used a slightly lower cut-off of LDL-C than traditionally defined (130 mg/dL or 3.37 mmol/L), as Indians are known to have lower LDL-C levels than Western populations [28,29]. Individuals with known CVD, uncontrolled blood pressure, clinically significant peripheral edema, impaired hepatic or renal function, ongoing steroid therapy, malignancy, known hypersensitivity to study drugs, or history of alcohol abuse or mental disorder were excluded from the study. Pregnant and lactating women were also excluded from the study. In addition, randomizing a small group of on-statin patients was also planned in a separate arm of the study. However, execution of this plan was discontinued later because of logistic reasons and also because such a randomization was not the primary objective of the study (Fig. 1).

### Study procedures

After enrolment, all participants underwent a general physical examination, 12-lead electrocardiogram, routine microscopic urine examination, and biochemical investigations including complete blood count, fasting lipid profile (total cholesterol [TC], LDL-C, high-density lipoprotein cholesterol [HDL-C], serum triglyceride), glycosylated hemoglobin, renal function tests (blood urea nitrogen, total bilirubin, and serum creatinine), and liver function tests (alanine transaminase, aspartate transaminase, and alkaline phosphatase). Blood samples were collected after an overnight fast for 8 to 12 h. All laboratory investigations were performed at a centralized facility (SRL Diagnostics, [www.srl.in](http://www.srl.in)).

After baseline assessments, each subject was assigned a unique subject number. On the randomization visit, the eligible subjects received one sealed envelope containing information regarding the subject number on the front and bottle number on the inner side of the envelope. The subjects were randomly assigned in a 1:1 ratio using these sealed envelopes to receive either RYR-NS, 600-mg capsule twice a day (n = 96) or a matching placebo (n = 95), which was administered for 12 wk in a double-blinded manner. All subjects were also advised lifestyle modifications and healthy diets by a certified dietitian according to their health status.

At 4 wk ( $\pm 4$  d) of study treatment, the subjects visited the trial site for clinical evaluation and blood collection for determining LDL-C and TC levels. Vital parameters were assessed and history was obtained about any possible adverse events for all the patients. Compliance with the study treatment was confirmed by questioning the subjects and collecting used bottles of the study medication before dispensing new bottles of RYR-NS or placebo capsules for the remaining study duration. The participants were also verbally questioned about their

**Table 1**  
Baseline clinical and biochemical characteristics of the study population

| Parameters   | RYR-NS (n = 92)                    | Placebo (n = 88)                   | P-value |
|--|------------------------------------|------------------------------------|---------|
| Age, years   | 47.0 $\pm$ 9.7                     | 47.4 $\pm$ 10.4                    | 0.77    |
| Male sex, n (%)  | 58 (63.0%)                         | 45 (51.1%)                         | 0.11    |
| Body weight, kg  | 67.3 $\pm$ 11.5                    | 69.6 $\pm$ 13.0                    | 0.21    |
| Diabetes, n (%)  | 31 (33.7)                          | 32 (36.4)                          | 0.71    |
| Body mass index, kg/m <sup>2</sup>                                     | 25.5 $\pm$ 4.2                     | 26.8 $\pm$ 4.7                     | 0.06    |
| Total cholesterol, mg/dL (mmol/L)                                      | 220.3 $\pm$ 32.4 (5.71 $\pm$ 0.84) | 221.2 $\pm$ 28.8 (5.73 $\pm$ 0.75) | 0.85    |
| Low-density lipoprotein cholesterol, mg/dL (mmol/L)                    | 146.7 $\pm$ 22.8 (3.80 $\pm$ 0.59) | 149.3 $\pm$ 21.5 (3.87 $\pm$ 0.56) | 0.42    |
| High-density lipoprotein cholesterol, mg/dL (mmol/L)                   | 42.1 $\pm$ 9.1 (1.09 $\pm$ 0.24)   | 40.0 $\pm$ 8.1 (1.04 $\pm$ 0.21)   | 0.10    |
| Non-high density lipoprotein cholesterol, mg/dL (mmol/L)               | 178.2 $\pm$ 30.9 (4.62 $\pm$ 0.80) | 181.3 $\pm$ 28.4 (4.70 $\pm$ 0.74) | 0.50    |
| Serum triglyceride, mg/dL (mmol/L)                                     | 163.0 $\pm$ 74.8 (1.84 $\pm$ 0.85) | 180.5 $\pm$ 67.1 (2.04 $\pm$ 0.76) | 0.10    |
| Glycosylated hemoglobin, %   | 6.3 $\pm$ 1.5                      | 6.4 $\pm$ 1.6                      | 0.59    |
| Blood urea nitrogen, mg/dL   | 10.8 $\pm$ 3.5                     | 10.8 $\pm$ 3.2                     | 0.98    |
| Serum creatinine, mg/dL  | 0.9 $\pm$ 0.2                      | 0.9 $\pm$ 0.2                      | 0.58    |
| Total bilirubin, mg/dL   | 0.56 $\pm$ 0.26                    | 0.46 $\pm$ 0.22                    | 0.01    |
| Alanine transaminase, U/L  | 48.1 $\pm$ 25.2                    | 44.0 $\pm$ 16.7                    | 0.19    |
| Aspartate transaminase, U/L  | 30.1 $\pm$ 30.3                    | 24.7 $\pm$ 9.5                     | 0.10    |
| Alkaline phosphatase, U/L  | 94.0 $\pm$ 20.6                    | 94.9 $\pm$ 24.9                    | 0.79    |
| Concomitant medications, n (%)   |                                    |                                    |         |
| Angiotensin converting enzyme inhibitors/angiotensin receptor blockers | 4 (4.3)                            | 1 (1.1)                            |         |
| Beta-blockers  | 1 (1.1)                            | 2 (2.3)                            |         |
| Oral hypoglycemic agents   | 3 (3.3)                            | 6 (6.8)                            |         |

**Table 2**  
Effect of treatment with RYR-NS on efficacy parameters

| Efficacy parameters, mg/dL (mmol/L)      | RYR-NS                     |                            | Placebo                    |                            | P-value*                   |                            |                 |
|--|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|-----------------|
|  | Baseline                   | 4 wk                       | 12 wk                      | Baseline                   | 4 wk                       | 12 wk                      |                 |
| Low-density lipoprotein cholesterol      | 146.7 ± 22.8 (3.80 ± 0.59) | 113.5 ± 30.1 (2.94 ± 0.78) | 102.6 ± 31.2 (2.66 ± 0.81) | 149.3 ± 21.5 (3.87 ± 0.56) | 138.5 ± 26.1 (3.59 ± 0.68) | 143.4 ± 29.1 (3.71 ± 0.75) | 0.0003 <0.0001  |
| Total cholesterol                        | 220.3 ± 32.4 (5.71 ± 0.84) | 179.0 ± 35.8 (4.64 ± 0.93) | 168.4 ± 34.8 (4.36 ± 0.90) | 221.2 ± 28.8 (5.73 ± 0.75) | 210.6 ± 36.1 (5.45 ± 0.93) | 205.4 ± 37.5 (5.32 ± 0.97) | <0.0001 <0.0001 |
| High-density lipoprotein cholesterol     | 42.1 ± 9.1 (1.09 ± 0.24)   | -                          | 46.1 ± 14.7 (1.19 ± 0.38)  | 40.0 ± 8.1 (1.04 ± 0.21)   | -                          | 43.5 ± 13.8 (1.13 ± 0.36)  | 0.01 0.10       |
| Non-high-density lipoprotein cholesterol | 178.2 ± 30.9 (4.62 ± 0.80) | -                          | 123.5 ± 33.4 (3.20 ± 0.87) | 181.3 ± 28.4 (4.70 ± 0.74) | -                          | 161.8 ± 37.8 (4.19 ± 0.98) | <0.0001 <0.0001 |
| Serum triglyceride                       | 163.0 ± 74.8 (1.84 ± 0.85) | -                          | 139.0 ± 79.6 (1.57 ± 0.90) | 180.5 ± 67.1 (2.04 ± 0.76) | -                          | 169.6 ± 74.8 (1.92 ± 0.85) | 0.22 0.008      |

All values are mean ± standard deviation

\* P-value is for overall comparison between the two groups and reflects time × treatment interaction.

adherence to lifestyle measures, including diet, and were counselled once again about the same.

At 12 wk (±4 days), all subjects underwent the same evaluations as at baseline. In addition, a detailed history about any adverse events that may have occurred since the last visit was also obtained.

#### Outcome measures

Primary efficacy outcome measures were absolute and percent change in LDL-C levels at wk 4 and 12. In addition, data were analyzed to determine the effect of RYR-NS on the following additional efficacy measures:

- Absolute and percent change in TC, triglyceride, HDL-C, and non-HDL-C levels at wk 12.
- Number of patients achieving desirable or above desirable levels of LDL-C and non-HDL-C, as per the 2014 U.S. National Lipid Association recommendations [30].

Primary safety measures were changes in renal and liver function parameters at wk 12, whereas the change in HbA1c at wk 12 was a secondary safety measure. In addition, the incidence and severity of adverse events was also recorded.

#### Statistical analysis

Standard descriptive analyses were performed to summarize the study parameters. All values are expressed as mean ± standard deviation or as counts and percentages. Baseline parameters in the two groups were compared using Student's independent samples *t* test or chi-square test as appropriate. Changes in quantitative parameters from baseline within each group were assessed using paired *t* test or repeated measures analysis of variance. Repeated measure modeling was also performed to evaluate the overall treatment effect on these parameters. McNemar test was used for evaluating changes in qualitative parameters. All statistical analyses were performed using the SAS statistical package, version 9.1 (SAS Institute Inc, Cary, NC, USA). A *P*-value of <0.05 was considered statistically significant.

#### Sample size estimation

Assuming an average standard deviation of 30 mg/dL (0.78 mmol/L) for LDL-C in the two groups, 72 subjects were required in each group to detect a postintervention difference of 15 mg/dL (0.39 mmol/L) with 85% power. Allowing a 20% drop-out rate, we included at least 90 subjects in each group.

## Results

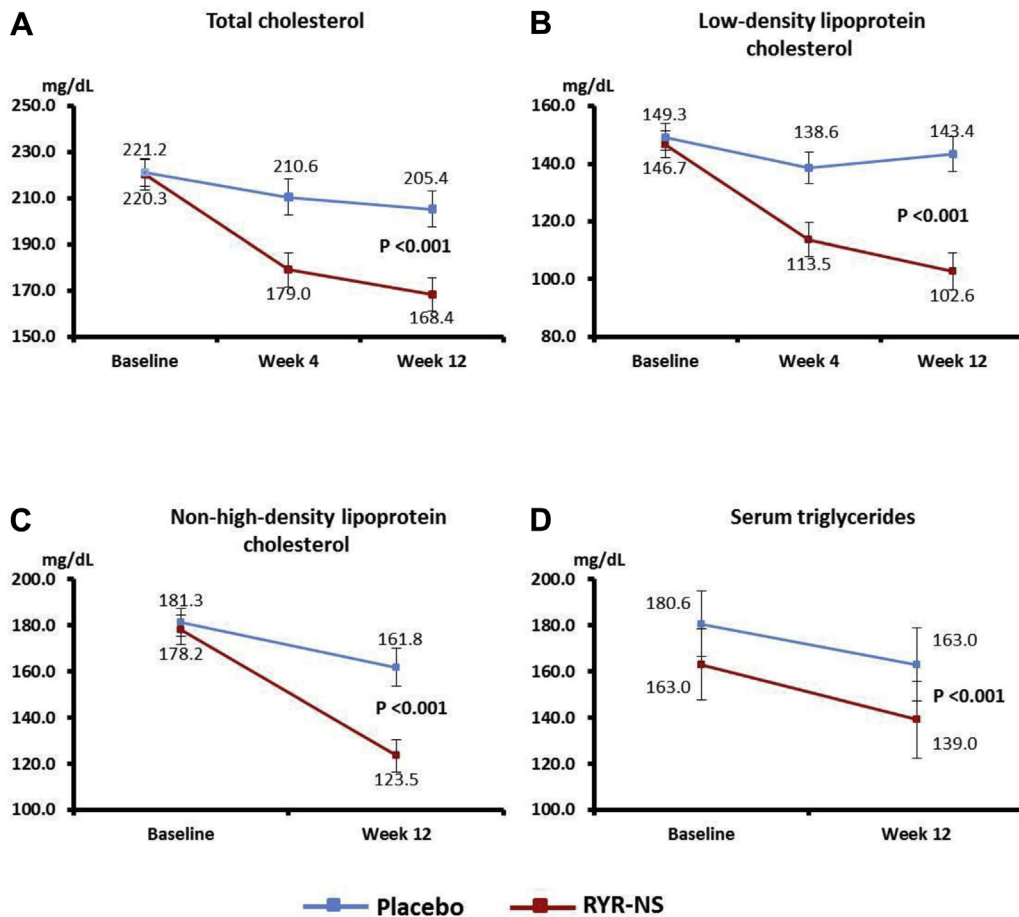
#### Baseline data

A total of 337 subjects were screened, out of which 191 subjects satisfying eligibility criteria were randomized to receive RYR-NS (96 subjects) or placebo (95 subjects). Of these, 11 patients (four in the RYR-NS group and seven in the placebo group) withdrew from the study, citing their unwillingness to continue their participation in a research project. The remaining 180 patients who completed the study were included in the analysis (Fig. 1).

The mean age of the subjects was 47.2 ± 10.0 y and 57% (103 of 180 subjects) were males. Sixty-three subjects (35.0%) had diabetes. Although the serum bilirubin level was slightly higher in the RYR-NS group (0.6 ± 0.3 mg/dL versus 0.5 ± 0.2 mg/dL for placebo, *P* = 0.01), there were no other significant differences between the two groups at baseline (Table 1). None of the participants were on any lipid lowering therapy (apart from the study formulation) and the use of other concomitant medications was also very infrequent.

#### Primary efficacy outcomes

Treatment with RYR-NS resulted in a significant reduction in LDL-C levels from 146.7 ± 22.8 mg/dL (3.80 ± 0.59 mmol/L) at baseline to 113.5 ± 30.1 mg/dL (2.94 ± 0.78 mmol/L) at wk 4 and 102.6 ± 31.2 mg/dL (2.66 ± 0.81 mmol/L) at wk 12



**Fig. 2.** Mean lipid levels in the two groups during the study period. All values represent mean with 95% confidence intervals. To convert to mmol/L, multiply by 0.0259 for cholesterol and 0.0113 for triglyceride. *P*-value is for overall comparison between the two groups and reflects time  $\times$  treatment interaction.

( $P < 0.0001$  for trend) (Table 2, Fig. 2). This translated into 22% reduction in LDL-C levels at wk 4 and 29% at wk 12 (Fig. 3). These changes were significantly greater than those observed in the placebo group (6.7% and 3.5% reduction at wk 4 and 12 respectively; all  $P$  values  $< 0.001$  for comparisons with the RYR-NS group).

#### Additional efficacy outcomes

RYR-NS resulted in significant reductions from baseline to wk 12 in the levels of TC ( $220.3 \pm 32.4$  mg/dL [ $5.71 \pm 0.84$  mmol/L] to  $168.4 \pm 34.8$  mg/dL [ $4.36 \pm 0.90$  mmol/L],  $-22.7\%$ ,  $P < 0.0001$ ), non-HDL-C ( $178.2 \pm 30.9$  mg/dL [ $4.62 \pm 0.80$  mmol/L] to  $123.5 \pm 33.4$  mg/dL [ $3.20 \pm 0.87$  mmol/L],  $-29.8\%$ ,  $P < 0.001$ ), and triglyceride ( $163.0 \pm 74.8$  mg/dL [ $1.84 \pm 0.85$  mmol/L] to  $139.0 \pm 79.6$  mg/dL [ $1.57 \pm 0.90$  mmol/L],  $-4.5\%$ ,  $P = 0.01$ ) (Table 2, Figs. 2 and 3), whereas HDL-C levels increased from  $42.1 \pm 9.1$  mg/dL ( $1.09 \pm 0.24$  mmol/L) to  $46.1 \pm 14.7$  mg/dL ( $1.19 \pm 0.38$  mmol/L), ( $+9.5\%$ ,  $P = 0.01$ ). Participants randomized to receive placebo also experienced reductions in TC and non-HDL-C levels (6.7% and 10.3%, respectively) but these changes were significantly lower than those observed in the RYR-NS group (both  $P < 0.0001$ ).

At baseline, only 20.7% patients in the RYR-NS group had LDL-C levels in the desirable ( $< 100$  mg/dL/ $2.59$  mmol/L) or above desirable (100–129 mg/dL/ $2.59$ – $3.34$  mmol/L) categories as per the U.S. National Lipid Association recommendations [30].

However, by the end of wk 12, this percentage increased to 84.8% ( $P < 0.0001$ ) with only 15.2% patients having borderline high (130–159 mg/dL or  $3.37$ – $4.12$  mmol/L), high (160–189 mg/dL or  $4.14$ – $4.90$  mmol/L), or very high ( $\geq 190$  mg/dL or  $4.92$  mmol/L) LDL-C levels (Fig. 4). In contrast, 14.8% patients had desirable or above desirable LDL-C levels at baseline in the placebo group, and this proportion increased to only 27.3% by the end of wk 12. Similarly, 39.1% patients in the RYR-NS group had desirable ( $< 130$  mg/dL or  $3.37$  mmol/L) or above desirable (130–159 mg/dL or  $3.37$ – $4.12$  mmol/L) non-HDL-C levels at baseline, which increased to 87.0% at wk 12 ( $P < 0.0001$ ). In the placebo group, the percentage of such patients increased from 23.9% at baseline to only 44.3% at the end of wk 12.

#### Safety and tolerability

RYR-NS was well tolerated during the 12 wk of treatment period and no study drug discontinuations occurred in any of the two groups. There were no clinically relevant changes in any of the safety parameters during the course of the study (Table 3). However, RYR-NS patients showed a small but statistically significant ( $P = 0.027$ ) decline in alanine transaminase levels from  $48.1 \pm 25.2$  U/L at baseline to  $42.4 \pm 15.8$  U/L, at wk 12 ( $P = 0.035$ ;  $P = 0.027$  for comparison with placebo) whereas total bilirubin level increased slightly in the placebo group from  $0.46 \pm 0.22$  mg/dL to  $0.51 \pm 0.21$  mg/dL ( $P = 0.028$ ;  $P = 0.08$  for comparison with the RYR-NS group). In the context of the

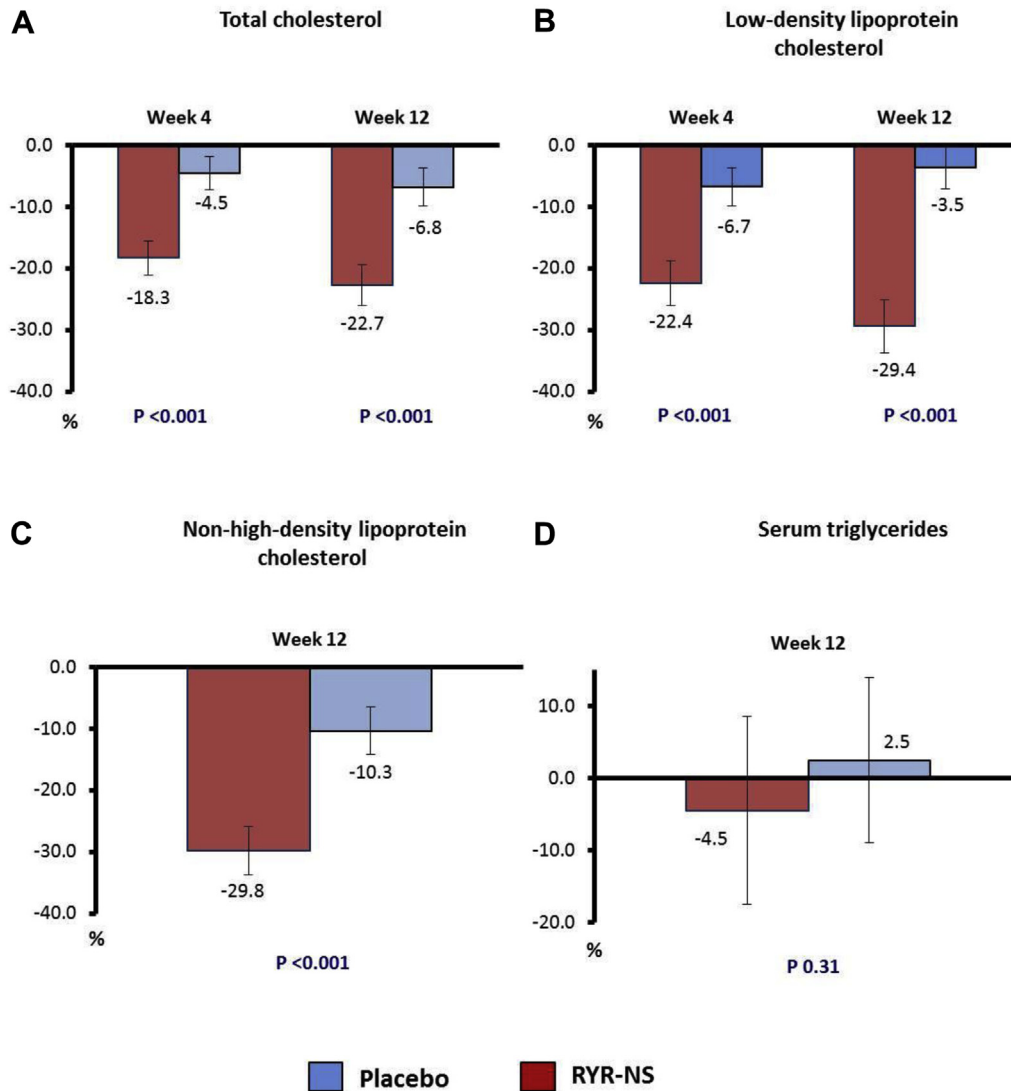


Fig. 3. Percent changes in various lipid parameters in the two groups during the study period. All values represent mean with 95% confidence intervals.

present study, these changes appear to be a result of the phenomenon of regression to the mean rather than of any actual change in liver functions.

Adverse events were very infrequent (gastric acidity/discomfort: RYR-NS 2, placebo 2; diarrhea/vomiting: RYR-NS 3, placebo 0; fatigability/body aches: RYR-NS 0, placebo 4) and were similar in the two groups (overall  $P = 0.91$ ). All adverse events were transient and none was severe. All adverse events were unrelated to the study treatment with the exception of one treatment-related event of gastric acidity in the placebo group.

## Discussion

This was the first randomized double-blind placebo-controlled study evaluating lipid lowering efficacy and short-term safety profile of a bioactive phytonutrient formulation in a primary prevention setting in India. We found that a 12-wk treatment with RYR-NS resulted in nearly 30% reduction in LDL-C and non-HDL-C levels, which is comparable with reductions seen with moderate-intensity statin therapy [31].

Additionally, no safety concerns were encountered with RYR-NS during the 12-wk treatment period.

### Lipid-lowering efficacy and the mechanisms of action of RYR-NS

RYR-NS consists of a mixture of food-derived bioactive ingredients (Appendix A) with distinct pharmacologic activities and can be standardized for predictable results. The qualitative and quantitative rationale for combining the specific ingredients in double zero vegetarian capsules with twice-daily dosing is proprietary to the investigational product, RYR-NS. The use of each ingredient in the combination is based on complementary and synergistic mechanisms of action in lipid metabolism and inflammatory pathways, bioavailability, and, importantly, stability in harsh weather conditions, which is common to low-income countries of the world.

RYR powder, which is the key ingredient, contains a milieu of monacolins, some of which are natural inhibitors of hydroxymethylglutarylcoenzyme A reductase, an enzyme also inhibited by statins. The lipid-lowering efficacy and safety of RYR has been

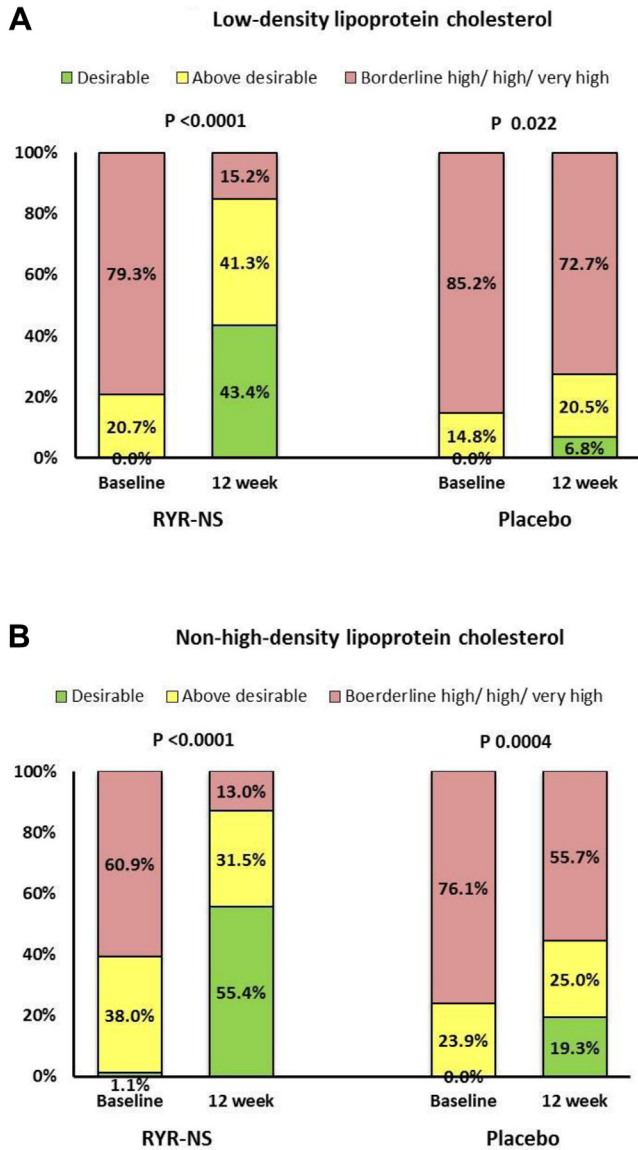


Fig. 4. Treatment effect on patient distribution in various lipid categories as per National Lipid Association recommendations. Please see text for further details.

evaluated in previous studies [21–24]. Of these, the only large study on RYR randomized 4870 participants to placebo or Xuezhikang, a partially purified extract of RYR. After a mean

follow-up for 4.5 y, treatment with Xuezhikang resulted in 45% reduction in the incidence of major adverse CV events [23]. Most of the other studies with RYR have been small but have consistently demonstrated its LDL-C-lowering efficacy [21]. RYR has also been evaluated in patients with statin intolerance. Becker et al. randomized 62 patients with dyslipidemia and a history of discontinuation of statin therapy because of myalgias to RYR or placebo for 24 wk [22]. RYR not only resulted in significant lowering of LDL-C, TC, and triglyceride levels, there was no increase in creatine phosphokinase levels and only two patients complained of myalgias as compared to one patient in the placebo group. Based on these published reports, the European Food Safety Authority has approved RYR as a food for maintenance of normal cholesterol levels [32]. However, RYR has heretofore never been tested in Indian subjects.

Other ingredients of RYR-NS also have beneficial lipid lowering or vascular effects. While niacin is known to increase HDL-C and lower LDL-C levels, folic acid reduces elevated homocysteine levels [25,26]. Grapeseed extract has antioxidant properties and may also lower LDL-C [27]. Black pepper seed powder (active principle, piperine) also has antioxidant effects and increases the bioavailability of various nutrients through a number of mechanisms [33].

However, for any combination of food-derived ingredients, the exact preparation needs to be tested independently for its efficacy and safety because of unknown interactions among different ingredients. In our study, the reduction in LDL-C levels achieved with RYR-NS was more than what has been demonstrated with RYR alone. This indicates a possible synergistic action between RYR and other ingredients of RYR-NS, especially niacin, folic acid, grape seed and black pepper seed powder. More importantly, despite a greater LDL-C-lowering effect, RYR-NS was not associated with any adverse events during the study period.

*Clinical relevance of the findings*

Statins are currently the most effective agents available for managing dyslipidemia and have been shown to reduce CV events in a wide variety of patient populations [10,25,31]. Accordingly, statins are the agents of choice in most patients requiring lipid-lowering therapy. However, it has been observed that even in those adequately treated with statins, the absolute risk of CV events remains high despite a significant relative risk reduction [5,8,9]. The pursuit to understand the mechanisms responsible for this high residual CV risk in statin-treated individuals has led to newer insights into the lipid-atherosclerosis relationship. Recent studies using non-statin therapies such as ezetimibe or proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors have shown that the residual CV risk can be

Table 3 Effect of treatment with RYR-NS on safety parameters

| Safety parameters                  | RYR-NS       |              |         | Placebo     |              |         | P-value* |
|------------------------------------|--------------|--------------|---------|-------------|--------------|---------|----------|
|                                    | Baseline     | 12 wk        | P-value | Baseline    | 12 wk        | P-value |          |
| Body mass index, kg/m <sup>2</sup> | 25.5 ± 4.2   | 25.5 ± 4.3   | 0.36    | 26.8 ± 4.7  | 26.8 ± 4.7   | 0.31    | 0.32     |
| Glycosylated hemoglobin, %         | 6.3 ± 1.5    | 6.2 ± 1.6    | 0.43    | 6.4 ± 1.6   | 6.3 ± 1.5    | 0.29    | 0.70     |
| Blood urea nitrogen, mg/dL         | 10.8 ± 3.5   | 11.1 ± 5.8   | 0.65    | 10.8 ± 3.2  | 10.7 ± 5.2   | 0.86    | 0.66     |
| Serum creatinine, mg/dL            | 0.9 ± 0.2    | 1.0 ± 0.4    | 0.18    | 0.9 ± 0.2   | 0.9 ± 0.3    | 0.28    | 0.48     |
| Total bilirubin, mg/dL             | 0.56 ± 0.26  | 0.54 ± 0.31  | 0.54    | 0.46 ± 0.22 | 0.51 ± 0.21  | 0.028   | 0.08     |
| Alanine transaminase, U/L          | 48.1 ± 25.2  | 42.4 ± 15.8  | 0.035   | 44.0 ± 16.7 | 45.2 ± 17.9  | 0.43    | 0.027    |
| Aspartate transaminase, U/L        | 30.1 ± 30.34 | 26.2 ± 15.46 | 0.27    | 24.7 ± 9.5  | 25.7 ± 10.25 | 0.36    | 0.18     |
| Alkaline phosphatase, U/L          | 94.0 ± 20.6  | 95.7 ± 25.8  | 0.55    | 94.9 ± 24.9 | 97.7 ± 30.3  | 0.33    | 0.77     |

All values are mean ± standard deviation

\* P-value is for overall comparison between the two groups.

reduced with further LDL-C lowering with these agents [18, 34–37]. At the same time, it has become increasingly evident now that not only the magnitude of LDL-C lowering is important but that the duration of exposure to elevated LDL-C levels is equally important [11,16,38]. Since atherosclerosis begins during childhood, initiating even moderate LDL-C lowering at an early stage of life can result in substantial reduction in the lifetime CVD risk. In contrast, lowering LDL-C levels when atherosclerosis has already become clinically overt may serve merely to stabilize existing plaques that can still progress to cause symptoms or disrupt to cause acute coronary syndromes, thus resulting in a high residual risk of events. The exceptionally low rates of CV events observed in patients carrying mutations in PCSK9 or other similar genes despite only modestly low LDL-C levels strongly support this notion [11]. Similar findings have also shown by Ference et al. in a large metaanalysis evaluating the impact of nine polymorphisms in six different genes involved in LDL-C metabolism [13]. It was found that lifelong exposure to low LDL-C levels as a result of these gene polymorphisms was associated with three-fold greater reduction in the risk of CVD per unit lower LDL-C level than that observed during treatment with a statin started later in life. These evidences clearly suggest that a primary prevention strategy that encourages maintaining low levels of LDL-C throughout one's lifetime has the potential to dramatically reduce the risk of CVD.

However, despite these encouraging evidences, it is not known how best to achieve effective LDL-C lowering from an early age. While adopting healthy lifestyle behaviors is certainly helpful and must be encouraged, it alone is not sufficiently effective because of general apathy toward these measures, as documented in the PURE (Prospective Urban Rural Epidemiology) study [39]. Although statins may be an effective option, they are limited by variable and largely poor uptake, with less than 5% of patients with CVD shown to be on regular statin therapy in low- and lower-middle-income countries [20]. The usage of statins in primary prevention settings is even lower, even in developed countries, where a vast majority of the patients are not on regular statin therapy [40]. The concerns of adverse effects and inherent psychological barrier against the lifetime use of a drug are amongst the major reasons responsible for such a low uptake of proven drugs like statins. These observations are corroborated by the PURE study data showing much lower usage of proven medical therapies by younger individuals as compared to older subjects [20]. Under these circumstances, the availability of a food-based therapeutic solution that appeals to the social and cultural sensibilities of the people from countries like India, China, and others where traditional medicine systems coexist could be very beneficial. If proven safe and effective, such a formulation could help improve lipid levels in these communities through potentially better adherence to these agents as compared to statin therapy. A functional food/bioactive phytonutrient formulation could also be potentially helpful in cases of actual statin intolerance [41] or when statins alone are not able to bring about desired LDL-C reduction, as there is currently lack of effective pharmacologic options that could be used either in place of or in combination with statin therapy.

Several food-based lipid lowering therapies have been tried in the past, showing variable results. For example, a metaanalysis of studies evaluating the role of spirulina supplementation documented significant LDL-C lowering effect [42], whereas no such changes were observed with other agents such as garlic and astaxanthin [43,44]. In the present study, we used RYR-NS and observed a significant LDL-C lowering effect. Importantly, while

none of the patients had LDL-C levels within desirable limits at baseline, the proportion increased to 43.4% with just 12 wk of treatment with RYR-NS. The impact on non-HDL-C levels was even greater with 55.4% of the subjects reaching desirable levels as compared to just 1.1% at the baseline. Substantial public health gains can be made if these reductions could be sustained over the long term. As part of ESSENS, large-scale, long-term studies are already being conceptualized to evaluate the impact of RYR-NS on clinical outcomes.

### Limitations

The main limitations of the present study included its relatively small sample size and short duration. As a result, it was not possible to determine the long-term safety of RYR-NS. However, while there is a need for a large, long-term, prospective, clinical study to evaluate the efficacy and safety of RYR-NS, it must be noted that the safety and efficacy of various food-derived ingredients of RYR-NS have already been evaluated in a number of small- and large-scale studies [21–24]. In fact, as outlined earlier, RYR has also been evaluated and found to be safe in a small group of statin intolerant patients. These results are reassuring since most of the side effects of various lipid lowering therapies, with the notable exception of statin-induced diabetes, are observed mainly during the initial few weeks of treatment.

The other major limitation of the present study was that only statin free patients were included. As a result, it could not be assessed whether RYR-NS could result in incremental reduction in LDL-C levels in patients already on statin therapy. Further studies are required to address this issue.

### Conclusion

This study demonstrates the efficacy and safety of RYR-NS in LDL-C and non-HDL-C lowering after 12 wk of treatment in Indians. A nearly 30% reduction in LDL-C levels observed at wk 12 with RYR-NS is comparable to reductions seen with moderate-intensity statin therapy. There were no safety/tolerability issues observed with RYR-NS. Further large-scale, long-term studies are being conceptualized to determine the impact of RYR-NS on treatment adherence, and thus, on clinical outcomes.

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**Appendix A**

Composition details of the study formulation RYR-NS

|   |   |
|---|---|
| <b>Scientific name of ingredients:</b> The Latin binomial name and common name  | Consists of a combination blend of following food-derived ingredients:  |
| <b>Manufacturer and brand name:</b> The proprietary product name (i.e., brand name) and the name of the manufacturer                | Manufacturer: PreEmptive Meds, Inc. USA   |
| <b>Ingredient extraction/manufacturing:</b> The part(s) of plant or botanical used to produce the product or extract & solvent used | Brand Name: PreLipid®   |
| <b>Serving size:</b> The dosage of the product, and how these were determined   | <ol style="list-style-type: none"> <li>1. Red yeast rice powder (<i>Monascus purpureus</i>)</li> <li>2. Grape seed extract powder (<i>Vitis vinifera</i>)</li> <li>3. Black pepper seed extract powder- (<i>Piper nigrum</i>)</li> <li>4. Niacinamide (Niacin)</li> <li>5. Vitamin B9 (Folic Acid)</li> </ol>   |
| <b>Dosage:</b> The content (e.g., as weight) of all ingredients and added materials   | <ol style="list-style-type: none"> <li>1. Red yeast rice (<i>Rice powder</i>). No solvent used</li> <li>2. Grape seed powder (<i>Seeds</i>). Water based solvent</li> <li>3. Black pepper powder- (<i>Seeds</i>) Water based solvent</li> <li>4. Niacinamide (Niacin) (<i>Synthetic</i>)</li> <li>5. Vitamin B9 (Folic Acid) (<i>Synthetic</i>)</li> </ol> <p>1. 600 mg twice-a-day in double-zero size Hydroxy Propyl Methyl Cellulose (HPMC) opaque red color capsules (Vegetarian, kosher &amp; halal certified)</p> <p>2. How dosage and duration is determined: Twice-daily dosage (one capsule in the morning and one at night) was based on half-life of food-based ingredients; quantity of standardized actives in each ingredient and based on proprietary knowledge gained from US clinical experience of eight years</p> <ol style="list-style-type: none"> <li>1. Red yeast rice powder: 400 mg;</li> <li>2. Grape seed extract powder: 35 mg;</li> <li>3. Black pepper powder: 10 mg;</li> <li>4. Niacinamide: 7 mg;</li> <li>5. Vitamin B9: 100 mcg;</li> <li>6. Excipients (starch, magnesium stearate) 100 mg</li> </ol> |
| <b>Placebo:</b> The rationale for the type of control or placebo used   | As the study was double-blinded, the placebo capsule used was double zero HPMC opaque red capsules and filled with 600 mg of maize starch.  |

**Appendix B. Participating sites**

1. Medanta- The Medicity, Gurgaon, Delhi NCR, India.
2. Fortis Escorts Hospital, Jaipur, Rajasthan, India.
3. Pai Research Center, Pune, Maharashtra, India.
4. Bhatia Hospital, Mumbai, Maharashtra, India.
5. Lifecare Hospital, Ahmedabad, Gujarat, India.
6. Apollo Hospital, Chennai, Tamil Nadu, India.
7. Singhvi Health Center, Chennai, Tamil Nadu, India.
8. Pace Research Center, Bangalore, Karnataka, India.
9. Pruthvi Heart Center, Ahmedabad, Gujarat, India.