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Cicero AF., D' Addato S, Borghi C. A Randomized, Double-Blinded, Placebo-Controlled, Clinical Study of the Effects of a Nutraceutical Combination (LEVELIP DUO®) on LDL Cholesterol Levels and Lipid Pattern in Subjects with Sub-Optimal Blood Cholesterol Levels (NATCOL Study). Nutrients. 2020;12(3127):1–10.

What We Know, Think We Know, or Are Starting to Know

Despite what you may encounter on the Googlweb of Things, we know - as much as we can know anything in biological sciences - that LDL-cholesterol causes, i.e., initiates and progresses, atherosclerosis and resulting cardiovascular disease [CVD] [1,2]. We also think that there may be clear thresholds at which atherosclerosis does not progress: at levels of LDL-C under 1.8mmol/L [70mg/dL] there is strong evidence that the progress of atherosclerosis may not only be arrested, but reversed [3,4].

Evidence is also starting to accumulate which suggests that for the prevention of CVD, in addition to 'lower is better', 'earlier is better', i.e., the lower LDL-C is maintained over the course of the lifespan, or the earlier the intervention in the disease process lowers LDL-C, the greater the extent of heart disease risk reduction [5,6]. The reason can be illustrated by the following graph:

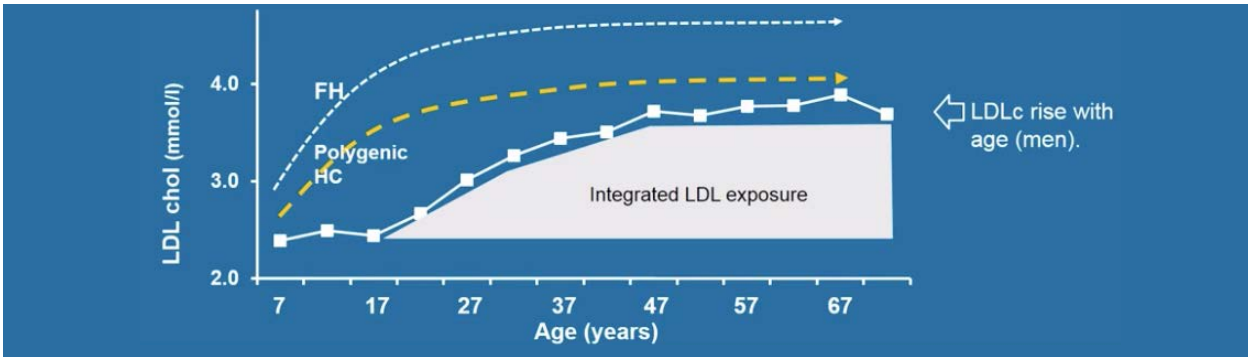

Figure *from Prof. Chris Packard presentation at the European Atherosclerosis Society Congress, 2018, depicting the concept of exposure to LDL as a cumulative, integrative exposure over the course of the lifespan. Atherosclerosis may develop from the second decade of life in otherwise healthy persons; the yellow dotted line depicts the curve for the integrated exposure in persons with genetically elevated cholesterol [not familial hypercholesterolemia], while the white dotted line depicts the extent in familial hypercholesterolemia [FH].*

Thus, the progression of atherosclerosis is a compounded effect of cumulative exposure to elevated LDL-C over the course of the lifespan. Strategies to prevent CVD, at both the individual and population level, remain focused on lifestyle in low-moderate risk persons, with an emphasis on diet, and targeting reduction of specific causal risk factors like LDL-C or hypertension [7]. The use of pharmacological means of lowering LDL-C come into play if reductions in LDL-C cannot be achieved by lifestyle means. Current recommendations for low risk individuals, i.e., individuals without other significant cardio-metabolic risk factors, are to maintain an LDL-C of <3.0mmol/L [116mg/dL] through diet and lifestyle.

There has been much interest in the capacity of specific 'functional foods' to maintain lower LDL-C, bolstered by the fact that many of these foods act through the same mechanism as drugs: the LDL-receptor*. Red yeast rice contains natural statin-like

compounds, known as monocolins, which inhibit the HMG-CoA reductase enzyme that statins target, reducing cholesterol synthesis in the liver [8]. Phytosterols are cholesterol-like molecules found in vegetable oils, nuts, grains, and legumes, that act by inhibiting intestinal absorption of cholesterol; they also inhibit recirculating endogenous biliary cholesterol, which is a key step in cholesterol elimination; phytosterols lower cholesterol by the same mechanism of action as the drug, ezetimibe [9]. Policosanol, a sugar alcohol derived from sugarcane wax, has been shown to reduce total cholesterol, but not LDL-C, although the mechanism of action remains unclear: it may inhibit cholesterol synthesis or enhance uptake of cholesterol in the liver [10].

Both phytosterols and red yeast rice of these compounds have been shown to reduce LDL-C levels by a range of ~10%-20%. The present study tested the effects of a combination patented supplement known as LEVELIP DUO® in participants with moderately elevated LDL-C.

*Geek Box: The LDL-Receptor

A major breakthrough in cardiovascular sciences was achieved in 1974, when Joseph Goldstein and Micheal Brown discovered that a genetic defect in the gene for the LDL-receptor was the underlying cause of familial hypercholesterolemia [FH]. The LDL-receptor is expressed in cells throughout the body, and its expression is responsible for taking up cholesterol which has been transported by LDL particles to body tissues, i.e., 'forward cholesterol transport'. By taking up cholesterol from LDL, the cholesterol content of LDL particles is reduced, and circulating levels of cholesterol are lowered. When the LDL-receptor is defective, as in conditions like FH, or its expression is suppressed through other factors, LDL cannot clear its cholesterol content and LDL remains in circulation carrying cholesterol, increasing the opportunity to cross into the arteries and deposit cholesterol in the artery wall. The discovery by Goldstein and Brown, which won them the Nobel Prize, also lead to the identification of the HMG-CoA-reductase enzyme as the rate-limiting enzyme in cholesterol synthesis, resulting in the development of statins to target the inhibition of this enzyme. By inhibiting HMGCR, cholesterol synthesis in the liver is reduced and the activity of the LDL-receptor is increased, resulting in greater clearance of LDL-C from the circulation, and lower LDL-C levels. This is a crucial point: every intervention shown to reduce CVD risk does so by ultimately resulting in increased expression of the LDL-receptor. The common pathway of increased LDL-C clearance from circulation is via upregulation of LDL-receptor activity. The mechanism through which this increased LDL-receptor activity is achieved varies from pathway to pathway, including inhibited intestinal absorption, inhibited cholesterol synthesis in the liver, or directly upregulated LDL-receptors. These respective pathways are influenced by genetic variants: NPC1L1 [intestinal absorption], HMGCR [hepatic cholesterol synthesis], and PCSK9 [LDL-receptor activity]. These pathways are the direct targets of various pharmacological interventions: bile acid sequestrates, e.g., ezetimibe [NPC1L1]; hepatic cholesterol synthesis, e.g., statins [HMGCR], and; increased LDL-receptor activity, e.g., PCSK9-inhibitors [PCSK9]. Oh, and this also happens to be the mechanism through which dietary saturated fat influence LDL-C levels and resulting CVD risk: saturated fats result in suppression of the LDL-receptor, impairing cholesterol clearance from the blood. The net result is the same, independent of the underlying mechanism, when the common pathway is increased LDL-C clearance via the LDL-receptor.

The Study

The "NATCOL Study" was a randomised, double-blind, placebo-controlled, parallel-arm intervention trial. 90 participants with moderately elevated LDL-C of 3.0-4.9mmol/L [115-190mg/dL] were enrolled; participants were not treated pharmacologically, were non-smokers, had a BMI of <32kg/m2, and triglycerides of <4.5mmol/L [<400mg/dL].

Prior to randomisation, all participants undertook a 2-week run-in period of following a Mediterranean diet [without excessive dairy or red meat products] and low intensity physical activity [20-30min brisk walk or cycle, 3-5 times per week]. After the two weeks, if LDL-C and triglycerides were in the range of the inclusion criteria, participants were randomised [by computer generation] to receive LEVELIP DUO® or a placebo, one tablet taken after dinner.

The LEVELIP DUO® intervention consisted of a combination of 800mg phytosterols, red yeast rice standardized to contain 5mg monacolins, 27mg niacin, and 10mg policosanols. The intervention lasted 8-weeks, and all participants underwent clinical measures at randomisation, after 4-weeks, and at the 8-weeks. The primary endpoint was changes in LDL-C levels from baseline to 8-weeks. Secondary outcomes included non-HDL-C, Apolipoprotein-B [ApoB], total cholesterol [TC], and other cardio-metabolic risk factors.

Results: Participants had a mean age of 51yrs, and 38 men and 47 women were randomised [19 men in the intervention and placebo groups, respectively, 24 and 23 women in the intervention and placebo groups, respectively]. Cardio-metabolic risk factors were similar between the intervention and placebo groups. Mean baseline LDL-C was 4.0mmol/L [155mg/dL] in the intervention group, and 4.1mmol/L [161mg/dL] in the placebo group. 43 and 42 participants in the intervention and placebo groups, respectively, were compliant [i.e., >80% of tablets taken].

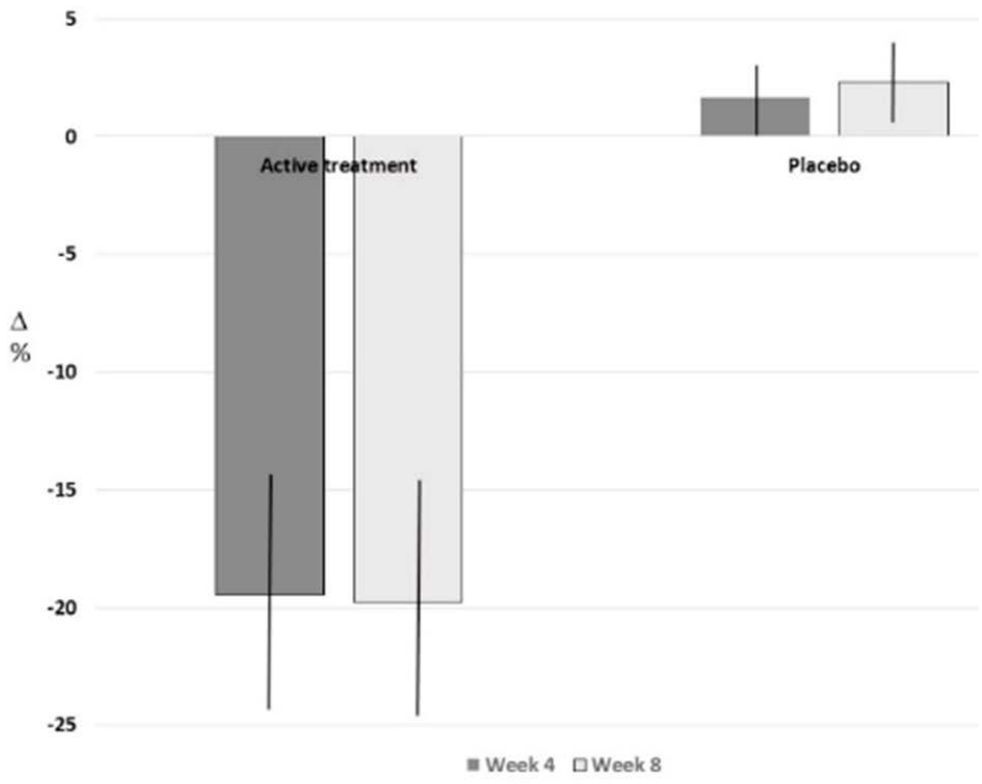

Figure *from paper illustrating the change in LDL-C after 4-weeks and 8-weeks in the intervention group (**left**) and placebo group (**right**). It appears from the data that the near maximum reduction of LDL-C was already achieved by 4-weeks, indicating acute effects of the supplement; it may also indicate a ceiling to treatment effect, however, as the trial was only 8-weeks in duration we do not know whether this is the case. Further studies will be required to elucidate the magnitude of the effect over the longer-term.*

>***LDL-C:*** After 8-weeks, LDL-C decreased by 19.8% in the intervention, a mean reduction of 0.8mmol/L [34.5mg/dL], with a 95% confidence interval range of 0.7-1.0mmol/L [27.9-41.1mg/dL]. Compared to the change in LDL-C in the placebo group, the between-group difference was 1.0mmol/L [39.2mg/dL], which was highly statistically significant.

>***non-HDL-C:*** After 8-weeks, non-HDL-C decreased by 0.8mmol/L [34.2mg/dL] in the intervention group; the between-group difference of 1.0mmol/L [41.7mg/dL] was highly statistically significant.

>***ApoB:*** After 8-weeks, ApoB decreased by 0.3mmol/L [15.2mg/dL] in the intervention group; the between-group difference of 0.4mmol/L [17.3mg/dL] was highly statistically significant.

>***TC:*** After 8-weeks, TC decreased by 0.8mmol/L [33.0mg/dL] in the intervention group; the between-group difference of 1.0mmol/L [41.2mg/dL] was highly statistically significant.

There were no statistically significant differences in body weight, waist circumference, blood pressure, or other cardio-metabolic secondary outcomes.

Critical Breakdown

Pros: The randomisation process was clear and appropriate. Both the intervention supplement and placebo were contained in indistinguishable tablets, maintaining blinding of participants and researchers. The supplement used purified extracts of monocolins [the red yeast rice compound] and policosanols, and the purity of the monocolins were verified by laboratory analysis. Compliance with taking both the intervention supplement and placebo was high at 94.4%. The trial required a minimum of 70 participants [35 per arm] for adequate statistical power, and 85 completed the study. The study arms were matched for sex and age.

Cons: There were no blood measures taken before the two week run-in, thus it is not possible to know what effect this period of diet and exercise leading into the intervention had on blood cholesterol levels. It is unclear what the placebo consisted of, presumably a cornstarch flour or similar product, but this is not mentioned. No data is presented in relation to diet, despite dietary advice being provided; with the known effects of certain Mediterranean diet characteristics - low saturated fat, high fibre, high polyphenols - diet may have been an important mediating factor influencing the outcomes. The study was quite short at 8-weeks, and it appears there was little change between 4 and 8-weeks, which could indicate a ceiling effect of the supplement [more under ***Interesting Finding***, below]. Given individual differences in LDL-C reduction is well-established, and suggested by the fact that the standard deviation was close to the mean value [indicating that individual values were not all close to the mean] it would have been useful to present a waterfall plot or scatter plot of the data to look at individual values, rather than just the bar chart of the mean. Bar charts are lazy.

Key Characteristic

The participants in this study were not high-risk, which is often the sample selected for interventions of lipid-lowering treatments. In fact, the participants had normal BMI, blood pressure, fasting glucose, and other cardio-metabolic risk factors. Only LDL-C was

moderately elevated, in a range of 3.0-4.9mmol/L [116-190mg/dL] considered by the European Atherosclerosis Society to warrant consideration of intervention to lower LDL-C. The otherwise healthy participant sample means that the effect of the supplemental intervention was specific to established, causal risk factors for CVD in LDL-C and ApoB, which is a marker for all circulating atherogenic lipoproteins in circulation, independent of other risk factors. This may be relevant for the 'earlier and lower is better' paradigm of treatment and prevention of CVD. Questions will remain, however, over whether the magnitude of reduction in this range, in otherwise healthy people, would translate into meaningful reductions in CVD events [more under ***Relevance***, below].

Interesting Finding

The changes in relevant blood cholesterol outcome measures were maximised at 4-weeks of the intervention. For example, LDL-C was 124.0mg/dL at 4-weeks and 122.6mg/dL at 8-weeks: TC, non-HDL-C, and ApoB were all similar. In a Chinese intervention using a purified red yeast rice extract in participants with moderately elevated cholesterol [(8)], maximum reductions in LDL-C were observed 6-8 weeks after randomisation: from 3.3mmol/L [129mg/dL] to 2.66mmol/L [103mg/dL], a 17.6% reduction similar in magnitude to the 19.8% observed in the present study. In a meta-analysis of the cholesterol lowering effect of phytosterols, an average LDL-C reduction of 12.1% was observed with a dose-response evident up to 2g/d phytosterols [(9)]. The dose in the supplement in this study of 800mg was stated to be the minimum effective dose for phytosterols; it may be that is was too low to exert any significant additive reduction in LDL-C. Nonetheless, the magnitude of reduction in this study is slightly higher than which has been observed for red yeast rice or phytosterols alone, which suggests some synergistic effect. This would be consistent with the distinct mechanisms of action of both, as phytosterols act similarly to ezetimibe, and red yeast rice similar to statins. Ultimately, it is not possible to elucidate the respective contributions of the ingredients of the supplement.

Relevance

The first caveat is that the effects of this trial will need to be replicated in order to demonstrate consistency in the effect of this combination 'functional food' supplement. However, with this caveat aside let' s take the results as they stand and contextualise the magnitude of effect relative to other interventions.

First, we know from the JUPITER trial that participants with already low LDL-C and ApoB, who achieved a reduction of LDL-C and ApoB of 1-50% from statin therapy, had a reduction in risk of CVD events of 39% [HR 0.61, 95% CI 0.44–0.83) for LDL-C and 50% [HR 0.50, 95% CI 0.38–0.65] for ApoB [(11)]. While the magnitude of risk reduction was linear, i.e., the lower the greater, the JUPITER data implies that the 19.8% reduction in LDL-C and 15% reduction in ApoB in the present study could at least be expected to have some reduction in actual 'hard' endpoints if sustained.

In support of this, in the Chinese red yeast rice intervention conducted over 4.5yrs, there was a 30% [RR 0.70, 95% CI 0.54-0.89] relative risk reduction for a CVD mortality following a 17.6% reduction in LDL-C levels [(8)]. In that study, the lowering of LDL-C occurred within 6-8 weeks of beginning the intervention, but the reduction was sustained over the course of 4.5yrs of the intervention. Thus, it is plausible that the effects observed in the present study over 8-weeks could be sustained over time to result in meaningful reductions in hard clinical endpoints. However, further research will need to confirm this.

Finally, a subgroup meta-analysis of 8 non-statin trials, all of interventions acting via increasing LDL-receptor expression, demonstrated that for each 1.0mmol/L [38.6mg/dL] reduction in LDL-C, CVD events were reduced by 25% [RR 0.75, 95% CI 0.66-0.86] [(12)]. Given this is the same mechanism of action through which phytosterols and red yeast rice ultimately act, there is sufficient plausibility to warrant further research in this 'functional food' supplement for reductions in CVD events, particular in population subgroups initially deemed low-risk. Based on the data by Silverman et al. [(12)] [figure below], the 0.8mmol/L [34.2mg/dL] reduction in LDL-C in the present could be predicted to lower risk of vascular events by between 15-20%.

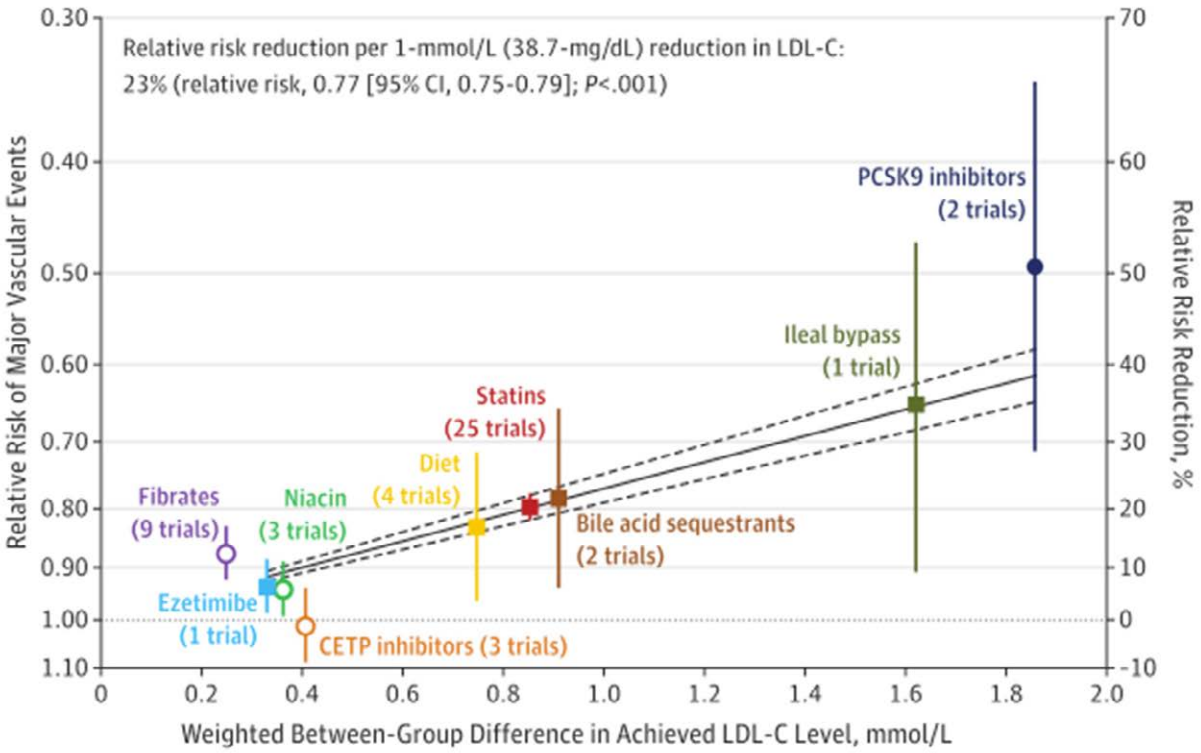

Figure *from Silverman et al. (12) indicating the relative risk of major vascular events per unit reduction of LDL-C through different means: statins, PSCK9 inhibitors, bile acid sequestrants, diet, etc. On the left Y-axis is the relative risk reduction of major vascular events; on the right Y-axis is the corresponding relative reduction as a percentage. The X-axis displays increasing, from left to right, levels of LDL-C reduction. Thus, as the line graph increases from left to right, this indicates that the risk reduction is becoming greater as the reduction in LDL-C increases.*

Application to Practice

The recent 2019 EAS guidelines for dyslipidemia state that purified red yeast rice with a daily dose of 5-10mg monocolins may be considered in individuals with elevated LDL-C who do not yet qualify for pharmacotherapy. However, it should be noted there is currently no regulation on the purity and quantity of monocolins for commercially available red yeast rice supplements. However, phytosterols are available on most supermarket shelves in the form of polyunsaturated spreads, for example FloraProActive, fortified with phytosterols at levels where the maximal dose of 2g/d may easily be obtained.

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