Clinical research

Effect of pravastatin on LDL particle concentration as determined by NMR spectroscopy: a substudy of a randomized placebo controlled trial

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Aim Recent data suggests that LDL particle concentration, as determined by Nuclear Magnetic Resonance (NMR) spectroscopy, may be associated with cardiovascular risk. We sought to determine the effect of randomization to pravastatin therapy on LDL particle concentration-NMR, among a primary prevention population.

Methods and results LDL particle concentration-NMR, LDL size-NMR, and standard chemical lipid parameters were measured at baseline and after 12 weeks among 500 individuals without overt coronary disease randomly allocated to pravastatin 40 mg (n=256) or placebo (n=244). Randomization to pravastatin therapy caused a 19% reduction in median LDL particle concentration-NMR at 12 weeks, as compared to a 4.2% increase among those randomized to placebo (P<0.001 for pravastatin group compared to placebo). Pravastatin therapy caused a median 24.9% reduction in LDL cholesterol measured chemically compared to a 0.9% increase in the placebo group (P<0.001). Pravastatin therapy did not cause a significant change in median LDL size-NMR (0.5% increase in pravastatin group vs 0.0% in placebo group; P=0.25). The change in LDL particle concentration with pravastatin correlated inversely with baseline LDL size (r=−0.24; P=0.001) such that the largest reduction in LDL particle concentration-NMR was among those with the smallest LDL size-NMR at baseline (median% change =21.4% for tertile 1 of LDL size, 19.9% for tertile 2, and 16.5% for tertile 3; P=0.03). In contrast, pravastatin-induced changes in LDL cholesterol did not correlate with baseline LDL size-NMR (r=−0.05; P=0.47).

Conclusion Among individuals without overt hyperlipidemia or known coronary artery disease, randomized allocation to pravastatin (40 mg) therapy for 12 weeks caused a reduction in LDL particle concentration-NMR, the magnitude of which was dependent on baseline LDL size-NMR.

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KEYWORDS
LDL particle concentration; Lipoproteins; Statin

Introduction

Nuclear Magnetic Resonance (NMR) spectroscopy provides a means for the quantification of low-density lipoprotein (LDL) particle concentration and size. Recent data suggests that LDL particle concentration-NMR may be associated with future cardiovascular risk. However, little is known about the effect of risk factor modification on LDL particle concentration-NMR and whether this lipoprotein parameter differs from traditional lipid measures. While certain statins have been reported to lower LDL particle concentration-NMR among patients
with dyslipidemia, data regarding the effect of HMG-CoA reductase inhibition on this lipoprotein risk factor among individuals without hyperlipidemia or known coronary disease are sparse. We sought to determine the effect of pravastatin on LDL particle concentration-NMR and size-NMR in a sub-study of the Pravastatin Inflammation/CRP Evaluation (PRINCE) study, a randomized trial of pravastatin therapy versus placebo.

Methods
As described elsewhere, PRINCE was a community-based, prospective, randomized trial of pravastatin 40 mg/day versus placebo carried out among 2882 men and women aged 21 years or older, who were free of statin use during the 6-month period prior to enrollment, and had no contraindications to statin therapy. Participants had known baseline LDL-Cholesterol levels of at least 130 mg/dl.

The current NMR based sub-study was conducted among a random selection of 500 participants in the PRINCE trial who had no prior history of cardiovascular disease, 256 of whom were randomized to pravastatin and 244 of whom were randomized to placebo. Blood samples obtained at baseline and 12 weeks were stored in liquid nitrogen until analysis. Total cholesterol, LDL cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels were measured in a Centers for Disease Control and Prevention-standardized laboratory.

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Lipoprotein subclass profiles were measured by proton NMR spectroscopy (LipoScience Inc., Raleigh, NC) as previously described. In brief, the NMR method uses the characteristic signals broadcast by lipoprotein subclasses of different size as the basis of their quantification. Each subclass signal emanates from the aggregate number of terminal methyl groups on the lipids contained within the particle. Cholesterol esters and triglycerides in the particle core each contribute three methyl groups and phospholipids and unesterified cholesterol in the surface shell each contribute two methyl groups. To a close approximation, the diameter of the particle determines the number of methyl groups present (and hence, the amplitude of the methyl NMR signal), irrespective of differences in lipid composition arising from, for example, variations in the relative amounts of cholesterol ester and triglyceride in the particle core, varying degrees of unsaturation of the lipid fatty acyl chains, or varying phospholipid composition. For this reason, the methyl NMR signal emitted by each subclass serves as a direct measure of the concentration of that subclass.

NMR spectra of each plasma specimen were acquired in duplicate at 47 °C on an automated 400 MHz lipoprotein analyzer and the lipid methyl signal envelope decomposed computationally to give the amplitudes of the contributing signals of 16 lipoprotein subclasses, among which are 4 LDL subclasses (IDL: 25±2 nm; large LDL: 22±0.7 nm; intermediate LDL: 20.5±0.7 nm; and small LDL: 19±0.7 nm). To obtain the conversion factors needed to relate these LDL signal amplitudes to particle concentrations, purified subclass standards were obtained and subjected to chemical lipid and NMR analysis. The subclass standards were isolated from a diverse group of normo- and dyslipidemic individuals by a combination of ultracentrifugation and agarose gel chromatography and characterized for size distribution by electron microscopy. Particle concentrations (nanomoles of particles per liter, nmol/L) were derived for each subclass standard by measuring the total core lipid concentration (cholesterol ester plus triglyceride) and dividing the volume occupied by these lipids by the calculated core volume per particle. Reported LDL particle concentrations (NMR) are the sums of the concentrations of the LDL subclasses (including IDL).

For all biochemical and NMR analyses, samples were handled in a fully blinded fashion such that all investigators had no knowledge of randomization status. Weighted average LDL particle sizes were computed as the sum of the diameter of each LDL subclass (excluding IDL) multiplied by its relative mass percentage as estimated from the amplitude of its methyl NMR signal. NMR LDL sizes are closely related to those estimated by gradient gel electrophoresis, but are uniformly smaller by approximately 5 nm, because they are referenced differently to diameters assessed by electron microscopy.

With 90% power and a significance level of 0.05, a sample size of 190 per group was required to detect a 10% change in LDL particle concentration with pravastatin therapy compared to placebo. We randomly selected 500 participants in the PRINCE trial who had no prior history of cardiovascular disease. Of these 500 randomly selected participants, 256 were in the pravastatin group, and 244 in the placebo group.

Baseline characteristics among the group randomized to pravastatin and the group randomized to placebo were compared with the chi squared test for categorical variables, and student’s t-test or Wilcoxon rank sum test for continuous variables. The median change and the median percent change in NMR measurements and lipid levels observed over the 12 week time course were also computed, and the significance of differences was evaluated between randomized treatment groups with the Wilcoxon rank sum test. Spearman correlation coefficients were computed to assess the association between on-treatment change in NMR parameters and lipid parameters. In order to further assess whether the change in LDL particle concentration-NMR observed with pravastatin was dependent upon baseline levels of LDL size-NMR, the group randomized to pravastatin were divided into tertiles according to baseline LDL size-NMR and the differences between the observed change in LDL particle concentration-NMR in these three groups was assessed by the Kruskal Wallace test. All P-values were two sided and a P value <0.05 was considered statistically significant.

Results

The baseline clinical characteristics of the study population, according to randomized assignment to placebo and pravastatin, are shown in Table 1. 244 of the study population were randomized to receive placebo and 256 to pravastatin therapy. There was a higher proportion of current smokers in the placebo group. Otherwise, the treatment groups did not differ with respect to age, gender, body mass index, diabetes, medication use, or lipid parameters. Baseline median levels of LDL particle concentration-NMR (1550.5 nmol/L) vs 1327.0 nmol/L (1318.5–1765.0), P=0.93) and LDL size-NMR (20.8 nm [20.4–21.2] vs 20.8 nm [20.4–21.2], P=0.82) were similar among those randomized to placebo and those randomized to pravastatin.

As shown in Table 2, randomization to pravastatin therapy caused a 19.0% reduction in median LDL particle concentration-NMR compared to a 4.2% increase in the placebo group (P<0.001). This compared with a median 24.9% reduction in chemically measured LDL cholesterol with pravastatin therapy compared to a 0.9% increase in
the placebo group ($P<0.001$). Pravastatin therapy did not cause a significant change in median LDL size-NMR compared to placebo (0.5% increase in pravastatin group compared to 0.0% in placebo group at 12 weeks, $P=0.25$). As expected, pravastatin therapy reduced levels of total cholesterol (median% change $=-18.3\%$; $P=0.001$ compared to placebo), and triglycerides (median% change $=-16.4\%$; $P=0.001$ compared to placebo), while causing a smaller increase in HDL levels (median% change $=+5.5\%$; $P=0.002$ compared to placebo).

Among those randomized to pravastatin therapy, the change in LDL particle concentration-NMR correlated significantly with the change in LDL cholesterol ($r=0.58$, $P<0.001$ and total cholesterol ($r=0.58$, $P<0.001$) and correlated inversely with the change in HDL-cholesterol ($r=-0.14$, $P=0.03$). The change in LDL particle concentration-NMR with pravastatin therapy did not differ significantly among current smokers and non-smokers (17.7% [8.9–26.6]% vs 19.1% [5.1–28.4%]; $P=0.92$).

Finally the change in LDL particle concentration-NMR with pravastatin therapy correlated inversely with baseline LDL size ($r=-0.24$; $P<0.001$). To further explore this relationship, we conducted further analyses whereby those randomized to pravastatin were divided into tertiles according to baseline LDL size-NMR. As shown in Table 3, pravastatin therapy caused the largest reduction in LDL particle concentration-NMR among those with the smallest LDL size-NMR at baseline (median% change $=21.4\%$ for tertile 1, 19.9% for tertile 2, and 16.5% for tertile 3; $P=0.03$). In contrast the pravastatin-induced change in LDL cholesterol did not correlate with baseline LDL size ($r=-0.05$; $P=0.47$).

### Discussion

In this substudy of a randomized placebo controlled trial, we found that randomization to pravastatin therapy for 12 weeks resulted in a 19% reduction in LDL particle concentration-NMR. This compared with a 24.9% reduction in LDL cholesterol with pravastatin therapy. Median LDL size-NMR did not change significantly with pravastatin therapy, but the reduction in LDL particle concentration-NMR with pravastatin therapy was most marked among those with smallest LDL size-NMR at baseline. In contrast the change in LDL cholesterol with pravastatin therapy was not related to baseline LDL size-NMR.

Apolipoprotein B-100 has been used as an estimate of LDL particle concentration and has been shown previously to correlate strongly with LDL particle concentration-NMR. In this regard, the observed reductions in LDL particle concentration-NMR with pravastatin therapy in this study are consistent with previous reports of reductions in apolipoprotein B-100 with statin therapy ranging from 13–25% among selected sub-populations. A previous report of the effect of pravastatin therapy on LDL particle concentration-NMR reported a 24% change in LDL particle concentration-NMR after 6 months of 20–40 mg of pravastatin among patients with documented coronary artery disease. Larger reductions in LDL particle concentration-NMR have been reported with atorvastatin therapy among 101 patients with atherogenic dyslipidemia and simvastatin therapy among 20 patients with mixed hyperlipidemia. That our data found somewhat smaller reductions may reflect that statins of different potency vary in their effects on LDL particle concentration, but also may reflect that our population was free of known cardiovascular disease and had lower baseline LDL particle concentrations.

We observed no change in median LDL size-NMR after 12 weeks of pravastatin therapy. This finding is consistent with the majority of other studies of the effect of statin therapy on LDL size assessed by other
<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Pravastatin</th>
<th>P value (Pravastatin vs placebo)</th>
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</thead>
<tbody>
<tr>
<td><strong>NMR measures</strong></td>
<td></td>
<td></td>
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<tr>
<td>LDL particle concentration (nmol/l)</td>
<td>1550.5</td>
<td>1591.0</td>
<td>+4.2%</td>
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<td>small LDL (nmol/L)</td>
<td>421.7</td>
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<td>intermediate LDL (nmol/L)</td>
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<td>large LDL (nmol/L)</td>
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<td>IDL (nmol/L)</td>
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<td>LDL size (nm)</td>
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<td><strong>Chemical lipid measures</strong></td>
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<tr>
<td>Total cholesterol (mg/dl)</td>
<td>230.55</td>
<td>231.63</td>
<td>231.82</td>
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<td>LDL-cholesterol (mg/dl)</td>
<td>142.47</td>
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<td>HDL-cholesterol (mg/dl)</td>
<td>39.46</td>
<td>40.53</td>
<td>40.16</td>
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<tr>
<td>Triglycerides (mg/dl)</td>
<td>155.0</td>
<td>147.0</td>
<td>152.5</td>
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</table>

Values are medians, except for total cholesterol, LDL-cholesterol, and HDL-cholesterol where values are mean. LDL=low-density lipoprotein; HDL=high-density lipoprotein.

*P values for the LDL subgroups refer to median absolute changes in LDL subgroup concentration in the pravastatin group compared to the placebo group.
techniques, although a recent study using NMR spectroscopy observed a small (1.5%) increase in LDL size with atorvastatin therapy among patients with hypertriglyceridemia. The magnitude of change in LDL particle concentration-NMR with pravastatin therapy did appear dependent on baseline LDL size-NMR with the greatest reduction observed among those with the smallest LDL size-NMR. This finding is also consistent with recent data from the Pravastatin Limitation of Atherosclerosis in the Coronaries (PLAC)-1 trial.

This study has a number of limitations. The proportion of current smokers was higher among those randomized to placebo than among those randomized to pravastatin. However, the effect of pravastatin on LDL particle concentration-NMR was similar among smokers and non-smokers, and thus we believe this chance occurrence is unlikely to have affected our results. We did not measure apolipoprotein B-100 levels in the PRINCE study. Finally, the use of frozen samples could theoretically have affected our results. Nonetheless, the observed levels of LDL particle concentration-NMR and size-NMR and lipid values are consistent with those reported in other studies.

In sum, randomized allocation to 40 mg of pravastatin therapy for 12 weeks caused a 19% reduction in LDL particle concentration-NMR among 500 patients without overt hyperlipidemia or known coronary artery disease. The magnitude of reduction in LDL particle concentration-NMR with pravastatin therapy was dependent on baseline LDL size-NMR. This association with LDL size was not observed for on treatment changes in LDL cholesterol. Further work is required to confirm the effects of other preventive dietetic and pharmacological interventions on LDL particle concentration-NMR.

References

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Effect of baseline tertile of LDL size-NMR on changes in LDL particle concentration-NMR with pravastatin therapy.</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL particle concentration</td>
<td>Baseline LDL size Tertile 1 (&lt;20.6 nm)*</td>
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<tr>
<td>Baseline (nmol/l)</td>
<td>1667.5 [1458.5–1963.0]</td>
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<tr>
<td>12 week (nmol/l)</td>
<td>1350.5 [1100.0–1569.5]</td>
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<tr>
<td>Median absolute change</td>
<td>349.0 [137.0–557.5]</td>
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<td>Median % change</td>
<td>21.4% [8.5–30.5%]</td>
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</tbody>
</table>

LDL=low-density lipoprotein.
*All values are median [interquartile range].